mm in diameter. In liquid media with glucose as the energy source growth occurs as light, uniform turbidity.

The type strain of *S. amylolytica* grows well in a glucose medium with rumen fluid replaced by trypticase and yeast extract. It also grows in a chemically defined medium containing glucose, CO_2/HCO_3 buffer, minerals, B vitamins, and acetate. Ammonium ions serve as the nitrogen source and cannot be replaced by amino acids or peptides. Sulfide serves as both the reducing agent and the sulfur source. Acetate (30 mM) is a highly stimulatory supplement even though the organisms produce acetate from glucose (Bryant and Robinson, 1962; Roberton and Bryant, unpublished data). During fermentation of glucose to succinate and acetate, a net uptake of CO_2 occurs. Of the substrates tested, only glucose, maltose, dextrin, and starch are fermented. Arabinose, xylose, fructose, cellobiose, lactose, sucrose, cellulose, inulin, xylan, glycerol, esculin, mannitol, lactate, amino acids, and peptides are not fermented. H_2S and indole are not produced. Gelatin is not liquefied. The Voges–Proskauer test is variable. Nitrate is not reduced.

Growth occurs at 30° and 37°C but not at 22° or 45°C. The final pH in poorly buffered, liquid glucose medium is 5.2–5.8. Good growth occurs at pH 6.5–7.0. The upper pH limit has not been determined.

S. amylolytica appears to be nonpathogenic for humans or animals. It occurs in the rumen of cattle fed diets containing roughage and some grain, where it is involved in fermentation of starch and its hydrolytic products. It is usually present as only a small proportion of the total viable bacteria in the bovine rumen (less than 6% of total). Whether *S. amylolytica* occurs in the rumen of ruminants other than cattle or in nonruminal ecosystems is not known.

Enrichment and Isolation Procedures

Succinimonas is isolated nonselectively in anaerobic roll tubes of RGCA medium from the rumen of cattle fed hay-grain diets. It constitutes only a small proportion of the colonies that develop after 3 d or longer of incubation at 37°C.

Maintenance Procedures

S. amylolytica strains can be maintained on stab-inoculated RGCA slants at −70°C for a year or more. They can also be preserved indefinitely by lyophilization.

Further Reading

Bryant, M.P., N. Small, C. Bouma and H. Chu. 1958. *Bacteroides ruminicola*, sp. nov. and *Succinimonas amylolytica*, gen. nov., species of succinic acid-producing anaerobic bacteria of the bovine rume. J. Bacteriol. *76*: 15–23.

Bryant, M.P. and I.M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. *84*: 605–614.

List of species of the genus Succinimonas

1. **Succinimonas amylolytica** Bryant, Small, Bouma and Chu 1958, 21.[AL]
   am.y.lo.1y′ti.ca. Gr. n. *amylum* fine meal, starch; Gr. adj. *lyticus* loosening, dissolving; M.L. fem. adj. *amylolytica* starch dissolving.
   The characteristics are as described for the genus.
   Occur in the bovine rumen.
   The mol% G + C of the DNA is: unknown.
   Type strain: ATCC 19206, DSM 2873, VPI 13846.
   GenBank accession number (16S rRNA): Y17599.

Order XIII. **"Enterobacteriales"**

En.te.ro.bac.te.ri.a′les. M.L. n. *enterobacterium* an intestinal bacterium; *-ales* ending to denote order; M.L. fem. n. *Enterobacteriales* the *enterobacterium* order.

Description is the same as for the family *Enterobacteriaceae.*

Type genus: **Escherichia** Castellani and Chalmers 1919, 941.

Family I. **Enterobacteriaceae** Rahn 1937, Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73; Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104

Don J. Brenner and J.J. Farmer III

En.te.ro.bac.te.ri.a′ce.ae. M.L. n. *enterobacterium* an intestinal bacterium; *-aceae* ending to denote a family; M.L. fem. pl. n. *Enterobacteriaceae* the family of the enterobacteria. Rahn's original derivation of the name *Enterobacteriaceae* is not certain. It may have come from his genus *Enterobacter*, or may have come from the root enterobacterium.

Gram-negative straight rods, 0.3–1.0 × 1.0–6.0 μm, except for *Arsenophonus*, which is 7–10 μm in length. Motile by peritrichous flagella, except for *Tatumella*, or nonmotile. Do not form endospores or microcysts; not acid-fast. Grow in the presence and absence of oxygen. Grow well on peptone, meat extract, and usually MacConkey's medium, except for *Calymmatobacterium* and insect symbionts, which have not been cultivated. Most grow well at 22–35°C; optimal growth and maximal biochemical capacity of a number of genera (*Yersinia*, *Hafnia*, *Xenorhabdus*, *Photorhabdus*, and many erwiniae) occurs at 25–28°C. Some grow on D-glucose as the sole source of carbon; some require vitamins and/or amino acids. **Chemoorganotrophic; having both a respira-**

tory and a fermentative metabolism. Acid and visible gas are often produced during fermentation of D-glucose, other carbohydrates and polyhydroxyl alcohols. Not halophilic. **Most are catalase-positive**, except for *Shigella dysenteriae* O group 1 and *Xenorhabdus*. **Most are oxidase negative**, except for *Plesiomonas*. **Most reduce nitrate to nitrite**, except *Saccharobacter fermentatus* (Yaping et al., 1990) and some strains of *Erwinia* and *Yersinia*.

The mol% G + C of the DNA is: 38–60 (63.5 for *S. fermentatus* (Yaping et al., 1990)).

Type genus: **Escherichia** Castellani and Chalmers 1919, 941. Designated type genus Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73.

Further descriptive information

The definition of the family circumscribes a large biochemically and genetically related group that shows substantial heterogeneity in its ecology, host range, and pathogenic potential for man, animals, insects and plants. The phylogenetic position of the family *Enterobacteriaceae* lies within the *Gammaproteobacteria*. Its nearest neighbors are the families *Alteromonadaceae*, *Vibrionaceae*, *Aeromonadaceae*, and *Pasteurellaceae*. Phylogenetic relationships of genera within the *Enterobacteriaceae* based on analysis of the 16S rRNA sequences of the type strains of the type species are shown in Fig. BXII.γ.189. Phenotypic characteristics useful in differentiating *Enterobacteriaceae* from its nearest phenotypic neighbors are shown in Table BXII.γ.191. The genera *Vibrio*, *Photobacterium*, and *Aeromonas* are oxidase positive, have polar flagella when grown in liquid media, and do not contain the enterobacterial common antigen—characteristics which distinguish them from *Enterobacteriaceae*. However, at least two *Vibrio* species (*V. metschnikovii* and *V. gazogenes*) are oxidase negative; strains of other species are oxidase negative or weakly positive; and, under certain conditions (often on solid media), members of these genera produce peritrichous flagella. Some *Aeromonas* species show higher DNA relatedness to *E. coli*, the type species of the type genus of *Enterobacteriaceae*, than that seen between several genera within the family. 16S rRNA gene sequence comparisons do not reveal any overlap between these families.

Biochemical variability and fastidiousness of a growing number of new species added to *Enterobacteriaceae*, the proposed inclusion in the family of the genus *Plesiomonas*, and the inclusion of *Calymmatobacterium* and a number of genera that are endosymbionts of insects has made a literal description of this family difficult. *Calymmatobacterium* is very fastidious, and cultivation is rarely successful—no strains are available in culture collections. The insect symbionts in the genera *Arsenophonus* (Gherna et al., 1991; Hypsa and Dale, 1997), *Buchnera* (Munson et al., 1991b; Clark et al., 1992), and *Wigglesworthia* (Aksoy, 1995b) have not been cultivated on bacteriological media, and therefore no biochemical profiles are available. These species can only be identified by their 16S rRNA gene sequences and perhaps by their host specificity. The description of the family therefore includes many traits for which there are exceptions. For example, *Plesiomonas*, a biochemically well-defined genus with a single species, is oxidase positive genus in an extremely large, oxidase negative family. Similarly, all cultivated species except *Erwinia chrysanthemi* contain enterobacterial common antigen, a trait that is specific for the family *Enterobacteriaceae*; two members of the family *Pasteurellaceae*, *Actinobacillus equuli* and *Actinobacillus suis*, also possess this antigen (Le Minor et al., 1972; Ramia et al., 1982; Böttger et al., 1987).

The relationships of many members of *Enterobacteriaceae* have been defined based on DNA relatedness (Wayne et al., 1987; Fox et al., 1992; Stackebrandt and Goebel, 1994). DNAs from species within most genera are at least 20% related to one another and to *Escherichia coli*, the type species of the type genus of the family. Notable exceptions are species of *Plesiomonas*, *Yersinia*, *Proteus*, *Providencia*, *Hafnia*, *Edwardsiella*, *Xenorhabdus*, and *Photorhabdus*, whose DNAs are usually 5–20% related to those of species from other genera. Most species have been analyzed by their 16S rDNA sequence. 16S rDNA sequence analysis is the method of choice for determining genera and higher taxa (Stackebrandt and Goebel, 1994); however, DNA relatedness can also be used to approximate evolutionary divergence within genera (Fig. BXII.γ.190).

The numbers of genera and species in the family have markedly increased during the past 25 years. There were 12 genera and 36 species in 1974 when the 8th edition of *Bergey's Manual of Determinative Bacteriology* was published (Buchanan and Gibbons, 1974). By 1984, when the 1st edition of *Bergey's Manual of Systematic Bacteriology* was published, the family contained 20 genera and 76 species (Krieg and Holt, 1984). The total rose to 30 genera and 107 species by the 9th edition of *Bergey's Manual of Determinative Bacteriology* in 1994 (Holt et al., 1994). In the classification used in this chapter, the family contains 44 genera and 176 named species. Unnamed published and unpublished genomospecies and presently undescribed groups undoubtedly include a number of new genera and species. A prediction of 225 or more species contained in 60 or more genera by the next edition of this *Manual* may be conservative.

Compared with the 1st edition of *Bergey's Manual of Systematic Bacteriology* and the 9th edition of *Bergey's Manual of Determinative Bacteriology*, the present volume contains proposed changes in classification, new genera, and new species. Taxonomic proposals since the last edition of the *Manual* are shown in Table BXII.γ.192 and discussed below and in the chapters describing individual genera. If there is any certainty with respect to the *Enterobacteriaceae*, it is that the family will continue to change and to pose a challenge to microbiologists in all specialties.

Plant, animal, and human disease *Enterobacteriaceae* are distributed worldwide. They are found in soil, water, fruits, meats, eggs, vegetables, grains, flowering plants and trees, and in animals from insects to man. Their pathogenicity for man and animals and economic importance, as well as their rapid generation time, ability to grow on defined media, and ease of genetic manipulation have made them the objects of intense laboratory study.

Many species are of considerable economic importance. Erwiniae and pectobacteria cause blight, wilt and soft-rot disease in corn, potatoes, apples, sugar cane, pineapples, and many other crops, often destroying substantial amounts of these crops (Starr and Chatterjee, 1972). For example, it has been estimated that plant pathogenic species causing soft rot diseases have caused at least $50 million of damage annually (Pérombelon and Kelman, 1980). The commercial and tropical fish industries are severely affected by the diseases caused by *Yersinia ruckeri* and species of *Edwardsiella* (Ewing et al., 1978; Shotts and Snieszko, 1976).

Salmonellosis in poultry and in eggs is a worldwide problem, both for poultry farmers and as a vehicle for human disease (Williams, 1965; Von Rockel, 1965; Hall, 1965; Mishu et al., 1994). Salmonellosis is also common in pigs, cows, horses, dogs, and cats (Barnes and Sorensen, 1975; Ewing, 1969). Stillbirths and wool damage in sheep are usually caused by salmonellae (Jensen,

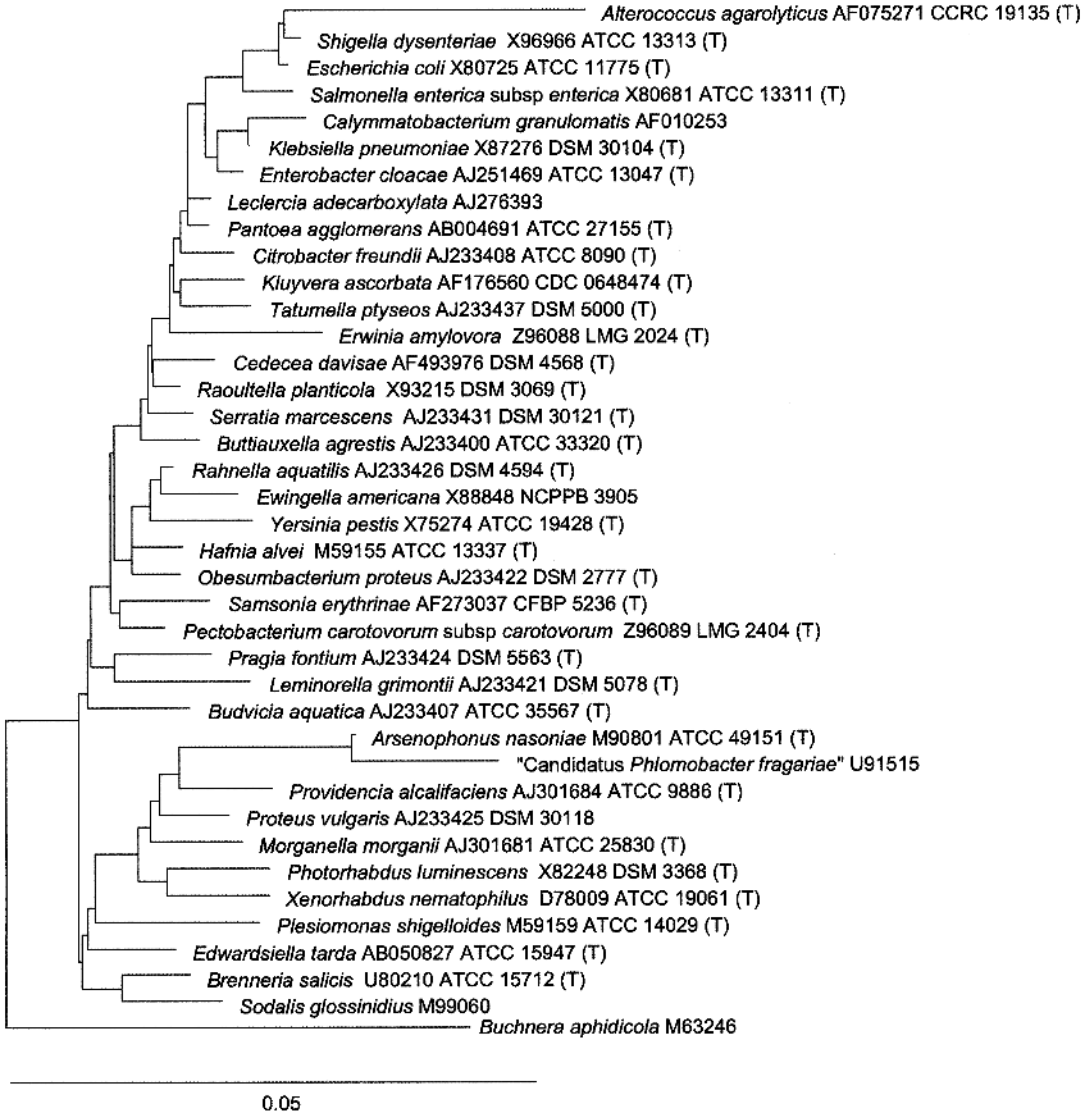


FIGURE BXII.γ.189. Phylogenetic relationships of the genera of the family *Enterobacteriaceae*. The distances in the tree were calculated using 1101 positions (the least-squares method, Jukes-Cantor model). (Courtesy T. Lilburn of the Ribosomal Database Project.)

1974). Enterotoxigenic *E. coli* strains are primarily responsible for diarrhea in lambs, piglets, and calves (Bruner and Gillespie, 1973). Klebsiellae and *Citrobacter freundii* are causes of bovine mastitis. Species of *Enterobacteriaceae* are responsible for numerous other animal infections. Examples include sexually transmitted uterine infections in horses caused by *Klebsiella pneumoniae*; infections in snakes, turtles and lizards caused by salmonellae; diarrheal and septicemic infection in rabbits, mink, and other rodents caused by yersiniae; and shigellosis in monkeys.

Salmonella serotype Typhi (*Salmonella typhi*) is the cause of typhoid fever, and *Yersinia pestis* causes bubonic and pneumonic plague. Many other *Enterobacteriaceae* are pathogenic for humans, causing a wide variety of diseases. These include diarrheal disease, usually transmitted by contaminated food or water; septicemia; respiratory disease; wound and burn infections; urinary tract infections; and meningitis. The causative agents of these diseases can be loosely divided into species that are normally pathogenic (*Salmonella*, *Shigella*, *K. pneumoniae*, *Y. pestis*, various serotypes of *E. coli*, and *Y. enterocolitica*) and species that cause disease under certain circumstances. Members of this second group are often referred to as opportunistic pathogens. The compromised host (for example, the malnourished, diabetic, immunosuppressed, catheterized, burn, cancer, respiratory, AIDS, or elderly patient) is vulnerable to nosocomial infections caused by opportunistic pathogens. *Enterobacteriaceae* have long been responsible for a substantial percentage of nosocomial infections in the United States (Jarvis et al., 1984), including urinary tract infections, surgical wound infections, lower respiratory infections, and bacteremias.

Salmonellae, *E. coli*, and shigellae are frequent causes of foodborne disease outbreaks (Beane et al., 1990), and *Yersinia enterocolitica* also causes foodborne disease. More recently, *E. coli* O157:H7, *Salmonella* serotype Typhimurium phage type 104 (definitive type 104, resistant to at least 5 antimicrobials), and *Salmonella* serotype Enteritidis phage type 4, have emerged as major foodborne pathogens (Boyce et al., 1995; Altekruse et al., 1997). *Salmonella* serotypes Typhimurium and Enteritidis are the two most prevalent causes of human salmonellosis in the United

TABLE BXII.γ.191. Some differential characteristics of the family *Enterobacteriaceae* and their nearest relatives[a]

Characteristic	*Enterobacteriaceae*	*Aeromonadaceae*	*Pasteurellaceae*	*Vibrionaceae*
Cell diameter, microns	0.3–1.5	0.3–1.0	0.2–0.4	0.3–1.3
Straight rods	+	D	+	D
Curved rods	−	D	−	D
Motility	D	+[b]	−	+[c]
Flagellar arrangement (liquid medium):				
Polar	−[d]	+		+
Lateral	+[d]	−		−
Oxidase test	−[e]	+[f]	+	+[f]
Sodium required or stimulatory for growth	−	−	−	D
Contain enterobacterial common antigen	+[g]	−	−[g]	−
Cells contain menaquinones[h]	D	D	−	D
Parasitic on mammals and birds	D	−[c]	+	−[c]
Heme and/or nicotinamide adenine dinucleotide required for growth	−	−	D	−
Plant pathogenicity	D	−	−	−
Organic nitrogen sources required	−[c]	−	+	−[c]

[a]Symbols: see standard definitions.

[b]Except *Ruminobacter*, *Tolumonas*, and certain biogroups of *Aeromonas salmonicida*.

[c]A few exceptions may occur.

[d]Except *Plesiomonas*, which has lateral polar flagella, and *Tatumella*, which may have polar, subpolar or lateral flagella.

[e]Except *Plesiomonas*.

[f]Except *Vibrio metschnikovii* and *Vibrio gazogenes* in *Vibrionaceae* and the genus *Tolumonas* in *Aeromonadaceae*.

[g]*Erwinia chrysanthemi* does not contain the antigen; *Actinobacillus equuli* and *Actinobacillus suis* contain the antigen.

[h]*Pasteurellaceae* do contain demethylmenaquinones but not menaquinones; ubiquinones may or may not be produced. *Enterobacteriaceae*, *Vibrionaceae*, and *Aeromonadaceae* may contain menaquinones, demethylmenaquinones, and ubiquinones.

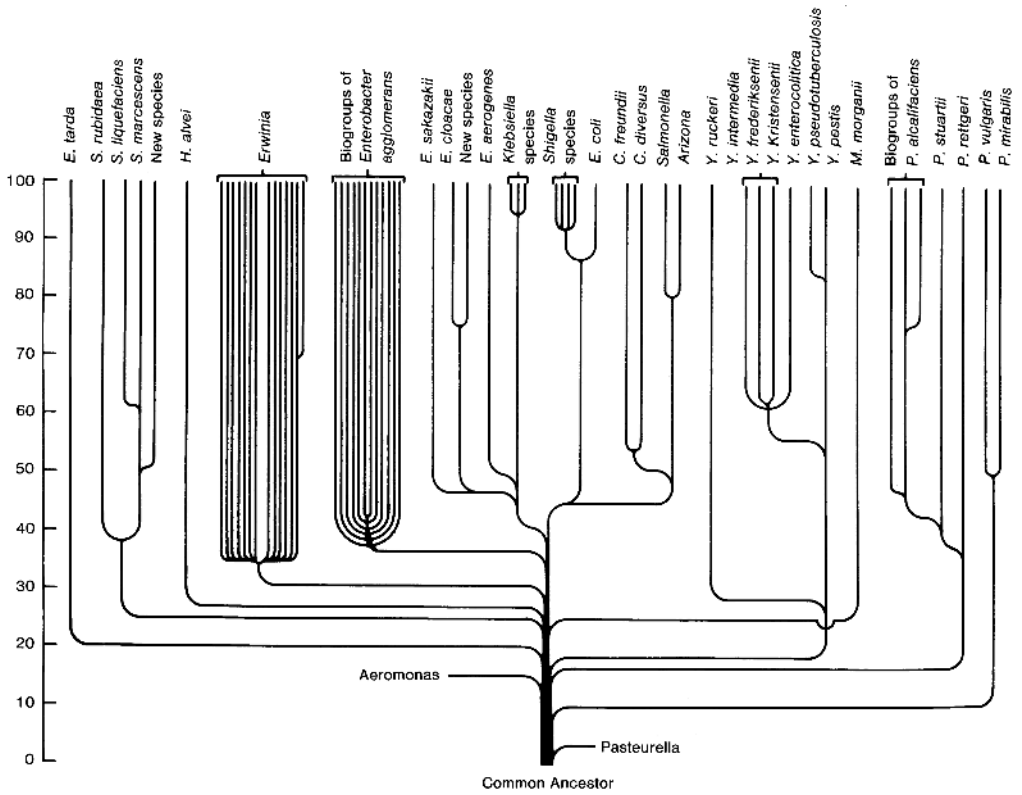


FIGURE BXII.γ.190. Divergence of *Enterobacteriaceae*. The ordinate is percentage of relatedness. This figure is a simplified attempt to depict relatedness of species of enterobacteria to other species. It assumes a common ancestor from which all of the organisms have diverged. The horizontal branches depict the degree of relatedness of the group of organisms to all organisms that have not yet branched. For example, *E. tarda* is 20% related to all organisms except *Aeromonas*, *Proteus*, *Providencia* and *Pasteurella*; *Citrobacter* species are 45% related to all species that branch above them and *C. diversus* and *C. freundii* were speciated at a point in time such that they are now 50% related.

TABLE BXII.γ.192. Comparison of the classification with that in the *Bergey's Manual of Systematic Bacteriology*, First Edition, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition[a]

Classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Alterococcus agarolyticus		NL
Arsenophonus nasoniae		*A. nasoniae*
Candidatus Arsenophonus triatominarum		NL
Brenneria alni	*Erwinia alni*	NL
Brenneria nigrifluens	*Erwinia nigrifluens*	*E. nigrifluens*
Brenneria paradisiaca	*Erwinia paradisiaca*	NL
Brenneria quercina	*Erwinia quercina*	*E. quercina*
Brenneria rubrifaciens	*Erwinia rubrifaciens*	*E. rubrifaciens*
Brenneria salicis	*Erwinia salicis*	*E. salicis*
Buchnera aphidicola		NL
Budvicia aquatica		*B. aquatica*
Buttiauxella agrestis		*B. agrestis*
Buttiauxella brennerae		NL
Buttiauxella ferragutiae	Enteric group 63	Enteric group 63
Buttiauxella gaviniae	Enteric group 64	Enteric group 64
Buttiauxella izardii		NL
Buttiauxella noackiae		NL
Buttiauxella warmboldiae		NL
Calymmatobacterium granulomatis	*Klebsiella granulomatis*	*C. granulomatis*
Cedecea davisae		*C. davisae*
Cedecea lapagei		*C. lapagei*
Cedecea neteri		*C. neteri*
Cedecea species 3		Cedecea species 3
Cedecea species 5		Cedecea species 5
Citrobacter amalonaticus	*Levinea amalonatica*	*C. amalonaticus*
Citrobacter braakii		NL
Citrobacter farmeri	*C. amalonaticus* biogroup 1	*C. amalonaticus* biogroup 1
Citrobacter freundii		*C. freundii*
Citrobacter gillenii	*Citrobacter* unnamed species 10	NL
Citrobacter koseri	*L. malonatica, C. diversus*	*C. diversus*
Citrobacter murliniae	*Citrobacter* unnamed species 11	NL
Citrobacter rodentium	*Citrobacter* unnamed species 9	NL
Citrobacter sedlakii		NL
Citrobacter werkmanii		NL
Citrobacter youngae		NL
Edwardsiella hoshinae		*E. hoshinae*
Edwardsiella ictaluri		*E. ictaluri*
Edwardsiella tarda	*E. anguillimortifera*	*E. tarda*
Enterobacter amnigenus		*E. amnigenus*
Enterobacter asburiae		*E. asburiae*
Enterobacter cancerogenus	*Enterobacter taylorae, Erwinia cancerogena*	*E. taylorae*
Enterobacter cloacae		*E. cloacae*
Enterobacter dissolvens	*Erwinia dissolvens*	*Enterobacter dissolvens*
Enterobacter gergoviae		*E. gergoviae*
Enterobacter hormaechei		*E. hormaechei*
Enterobacter kobei		NL
Enterobacter nimipressuralis	*Erwinia nimipressuralis*	*Enterobacter nimipressuralis*
Enterobacter pyrinus		NL
Enterobacter sakazakii	yellow-pigmented *Enterobacter cloacae*	*E. sakazakii*
Erwinia amylovora		*E. amylovora*
Erwinia aphidicola		NL
Erwinia billingiae		NL
Erwinia mallotivora		*E. mallotivora*
Erwinia persicina	*"E. nulandii"*	*E. persicinus*
Erwinia psidii		*E. psidii*
Erwinia pyrifoliae		NL
Erwinia rhapontici	*Pectobacterium rhapontici*	*E. rhapontici*
Erwinia tracheiphila		*E. tracheiphila*
Escherichia blattae		*E. blattae*
Escherichia coli		*E. coli*
Escherichia fergusonii		*E. fergusonii*
Escherichia hermannii		*E. hermannii*
Escherichia vulneris		*E. vulneris*
Ewingella americana		*E. americana*
Hafnia alvei		*H. alvei*
Klebsiella mobilis	*Enterobacter aerogenes*	*Enterobacter aerogenes*
Klebsiella oxytoca		*K. oxytoca*
Klebsiella ornithinolytica	*Raoultella ornithinolytica*	NL
Klebsiella planticola	*Raoultella planticola*	*K. planticola*
Klebsiella pneumoniae subsp. *ozaenae*	*K. ozaenae*	*Klebsiella pneumoniae* subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*		*Klebsiella pneumoniae* subsp. *pneumoniae*

(*continued*)

TABLE BXII.γ.192. *(cont.)*

Current classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Klebsiella pneumoniae subsp. *rhinoscleromatis*	*K. rhinoscleromatis*	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*
Klebsiella terrigena	*Raoultella terrigena*	*K. terrigena*
Kluyvera ascorbata		*K. ascorbata*
Kluyvera cochleae	*Enterobacter intermedius*[b]	*Enterobacter intermedium*
Kluyvera cryocrescens		*K. cryocrescens*
Kluyvera georgiana	*Kluyvera* species 3	*Kluyvera* species 3
Leclercia adecarboxylata	*Escherichia adecarboxylata*, *Enterobacter agglomerans* DNA group XI	*L. adecarboxylata*
Leminorella grimontii		*L. grimontii*
Leminorella richardii		*L. richardii*
Leminorella species 3		
Moellerella wisconsensis		*M. wisconsensis*
Morganella morganii subsp. *morganii*	*Proteus morganii*	*M. morganii*
Morganella morganii subsp. *sibonii*	*Proteus morganii*	*M. morganii*
Obesumbacterium proteus	*Hafnia protea*	*O. proteus*
Pantoea agglomerans	*Enterobacter agglomerans* DNA group XIII, *Erwinia herbicola*, *Erwinia milletiae*	*Enterobacter agglomerans*
Pantoea ananatis	*Pantoea ananas*, *Erwinia ananas*, *Enterobacter agglomerans* DNA group VI, *Erwinia uredovora*	*Erwinia ananas*
Pantoea citrea		NL
Pantoea dispersa	*E. agglomerans* DNA group III, phenon 8	*P. dispersa*
Pantoea punctata		NL
Pantoea stewartii subsp. *indologenes*		NL
Pantoea stewartii subsp. *stewartii*		*Erwinia stewartii*
Pantoea terrea		NL
Pectobacterium cacticida	*Erwinia cacticida*	*Erwinia cacticida*
Pectobacterium carotovorum subsp. *atrosepticum*	*Erwinia atroseptica*, *E. carotovora* subsp. *atroseptica*	*Erwinia carotovora* subsp. *atroseptica*
Pectobacterium carotovorum subsp. *betavasculorum*	*Erwinia carotovora* subsp. *betavasculorum*	*Erwinia carotovora* subsp. *betavasculorum*
Pectobacterium carotovorum subsp. *carotovorum*	*Erwinia carotovora* subsp. *carotovora*	*Erwinia carotovora* subsp. *carotovora*
Pectobacterium carotovorum subsp. *odoriferum*	*Erwinia carotovora* subsp. *odorifera*	NL
Pectobacterium carotovorum subsp. *wasabiae*	*Erwinia carotovora* subsp. *wasabiae*	*Erwinia carotovora* subsp. *wasabiae*
Pectobacterium chrysanthemi	*Erwinia chrysanthemi*	*Erwinia chrysanthemi*
Pectobacterium cypripedii	*Erwinia cypripedii*	*Erwinia cypripedii*
Candidatus Phlomobacter fragariae		NL
Photorhabdus luminescens subsp. *luminescens*	*Xenorhabdus luminescens*	*X. luminescens*
Photorhabdus luminescens subsp. *akhurstii*		NL
Photorhabdus asymbiotica		NL
Photorhabdus temperata		NL
Plesiomonas shigelloides[b]		*P. shigelloides*[b]
Pragia fontium		*P. fontium*
Proteus mirabilis		*P. mirabilis*
Proteus myxofaciens		*P. myxofaciens*
Proteus penneri		*P. penneri*
Proteus vulgaris		*P. vulgaris*
Providencia alcalifaciens		*P. alcalifaciens*
Providencia heimbachae		*P. heimbachae*
Providencia rettgeri		*P. rettgeri*
Providencia rustigianii		*P. rustigianii*
Providencia stuartii		*P. stuartii*
Rahnella aquatilis		*R. aquatilis*
Rahnella genomospecies 2		NL
Rahnella genomospecies 3		NL
Saccharobacter fermentatus		NL
Salmonella bongori	*Salmonella* subsp. *bongori*, *Salmonella* subsp. V	*"S. bongori"*[c], *Salmonella bongori*[d]
Salmonella enterica subsp. *arizonae*	*S. arizonae* (monophasic), *Salmonella* subsp. IIIa	*Salmonella arizonae*[c], *S. choleraesuis* subsp. *arizonae*[d]
Salmonella enterica subsp. *diarizonae*	*S. arizonae* (diphasic), *Salmonella* subsp. IIIb	*S. choleraesuis* subsp. *diarizonae*[d]
Salmonella enterica subsp. *enterica* Choleraesuis	*Salmonella* subsp. I	*Salmonella choleraesuis*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Choleraesuis[d]
Salmonella enterica subsp. *enterica* Enteritidis		*Salmonella enteritidis*[c]
Salmonella enterica subsp. *enterica* Gallinarum		*"Salmonella gallinarum"*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Gallinarum[d]
Salmonella enterica subsp. *enterica* Paratyphi A		*"Salmonella paratyphi-A"*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Paratyphi A[d]
Salmonella enterica subsp. *enterica* Paratyphi B		NL

(*continued*)

TABLE BXII.γ.192. *(cont.)*

Current classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Salmonella enterica subsp. *enterica* Paratyphi C		NL
Salmonella enterica subsp. *enterica* Typhi		*Salmonella typhi*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Typhi[d]
Salmonella enterica subsp. *enterica* Typhimurium		*Salmonella typhimurium*[c]
Salmonella enterica subsp. *houtenae*	*Salmonella* subsp. IV	*"Salmonella houtenae"*[c] *S. choleraesuis* subsp. *houtenae*[d]
Salmonella enterica subsp. *indica*	*Salmonella* subsp. V	*S. choleraesuis* subsp. *indica*[d]
Salmonella enterica subsp. *salamae*	*Salmonella* subsp. II	*"Salmonella salamae"*[c], *S. choleraesuis* subsp. *salamae*[d]
Serratia entomophila		*S. entomophila*
Serratia ficaria		*Serratia ficaria*
Serratia fonticola		*S. fonticola*
Serratia grimesii		*S. grimesii*
Serratia liquefaciens		*S. liquefaciens*
Serratia marcescens		*S. marcescens*
Serratia odorifera		*S. odorifera*
Serratia plymuthica		*S. plymuthica*
Serratia proteamaculans subsp. *proteamaculans*		*S. proteamaculans* subsp. *proteamaculans*
Serratia proteamaculans subsp. *quinovora*		*S. proteamaculans* subsp. *quinovora*
Serratia rubidaea		*S. rubidaea*
Shigella boydii		*S. boydii*
Shigella dysenteriae		*S. dysenteriae*
Shigella flexneri		*S. flexneri*
Shigella sonnei		*S. sonnei*
Sodalis glossinidius		NL
Tatumella ptyseos		*T. ptyseos*
Trabulsiella guamensis		NL
Wigglesworthia glossinidia		NL
Xenorhabdus beddingii	*X. nematophilus* subsp. *beddingii*	*X. beddingii*
Xenorhabdus bovienii	*X. nematophilus* subsp. *bovienii*	*X. bovienii*
Xenorhabdus japonica		NL
Xenorhabdus nematophila		*X. nematophilus*
Xenorhabdus poinarii	*X. nematophilus* subsp. *poinarii*	*X. poinarii*
Yersinia aldovae		*Y. aldovae*
Yersinia bercovieri		*Y. bercovieri*
Yersinia enterocolitica		*Y. enterocolitica*
Yersinia frederiksenii		*Y. frederiksenii*
Yersinia intermedia		*Yersinia intermedia*
Yersinia kristensenii		*Y. kristensenii*
Yersinia mollaretii		*Y. mollaretii*
Yersinia pestis		*Y. pestis*
Yersinia pseudotuberculosis		*Y. pseudotuberculosis*
Yersinia rohdei		*Y. rohdei*
Yersinia ruckeri		*Y. ruckeri*
Yokenella regensburgei	*Koserella trabulsii*, *Hafnia* hybridization group 3, Enteric group 45, Enteric group 45, NIH (Japan) biogoup 9	*Y. regensburgei*

[a]NL, not listed.

[b]*Enterobacter intermedius* and *Kluyvera cochleae* have identical 16S rRNA sequences.

[c]Name used in *Bergey's Manual of Systematic Bacteriology*, First Edition, 1984.

[d]Name used in *Bergey's Manual of Determinative Bacteriology*, Ninth Edition, 1994.

States and in the United Kingdom. Outbreaks due to *E. coli* O157:H7 have occurred in the United States, Europe, Asia, and Africa. In the United States, this organism is the leading cause of hemolytic uremic syndrome, the main cause of acute kidney failure in children (Boyce et al., 1995). *Enterobacteriaceae* are also significant causes of waterborne disease outbreaks.

Maintenance Procedures The following procedures have been used for many years in the Enteric Reference Laboratory, CDC, and seem to work well for all the genera and species of *Enterobacteriaceae*. Many other procedures have been described in the literature, but there have been few, if any, good comparative studies.

Long term preservation of cultures. Compared to many other organisms, strains of *Enterobacteriaceae* are simple to grow and maintain. However, strains will die if they are allowed to dry on plating media, agar slants or in "deeps." This die off is mainly due to drying. Freezing and storage at low temperature is the method of choice for long term preservation and maintenance. An alternative method is lyophilization and storage at 4°C, which is used routinely, with good results, by the American Type Culture Collection.

Preparation of a permanent frozen stock of the "whole culture." All important cultures of *Enterobacteriaceae* should be frozen and stored in liquid nitrogen. If liquid nitrogen storage is not available, they can be stored in a laboratory freezer at −70°C, or even in a "home freezer" at −10 to −20°C. The lowest tem-

perature available should be used for long term storage. The culture to be maintained is first inoculated heavily and grown on a blood agar plate for 24 h (or longer if it grows slowly). Growth from the heavy area of growth (designated as the "whole culture," as opposed to a single colony) is removed with a cotton swab, and a very heavy suspension is made in 10% skim milk (a variety of other suspending media are also in general use). One ml of this suspension is then removed to a sterile plastic freezer vial. A permanent marking pen is used to write the genus and species names and the strain number. The vial is then frozen in an alcohol bath kept in the freezer. Vials are then transferred to a 100-compartment storage box and stored at the lowest temperature available.

Preparation of a "working stock" culture of the single colony. This procedure is recommended for cultures that are used frequently, since it avoids the inconvenience of repeatedly going to a freezer. Growth from the top of a single, isolated colony is touched and stabbed to the bottom of a 13 × 100 mm screw-cap tube of working stock medium. The culture is grown overnight and checked to insure that the tube has good growth. The tube is sealed by closing the cap tightly. These working stocks are convenient for daily use. A small amount of growth is removed with a loop and transferred to a plate or tube of a good growth medium. After incubation, the culture is ready to use. The sealing method described above does not give an air-tight fit, thus water from the semi-solid medium will begin to evaporate at a rate dependent on the tightness of the fit. Drying is not a problem for many months, but eventually the viable count of the culture may begin to drop. To avoid this problem, two alternative sealing methods are used. A small volume (about 0.3 ml) of sterile mineral oil can be added to cover the surface (about 3 mm above the interface), which prevents evaporation of the medium. This works extremely well in preventing evaporation, but the mineral oil is messy and must be considered when the culture is transferred. A second method to prevent drying is to insert a number "000" white rubber stopper into the neck of the screw-cap tube to seal it. Cultures are conveniently stored at room temperature in the dark in 40-compartment divided boxes. Many species of *Enterobacteriaceae* survive for decades with this method, but some species die more quickly than others. To avoid the death of cultures, they should be frozen in addition to being stored at room temperature.

Differentiation of genera and species of *Enterobacteriaceae*

Genera and species of the family *Enterobacteriaceae* have traditionally been differentiated based on biochemical tests. Unfortunately, the media and tests are not completely standardized, and few laboratories use exactly the same formulations or procedures. Even with these variables, this approach usually results in correct identifications of the common species of *Enterobacteriaceae*. Biochemical reactions for *Enterobacteriaceae* are presented in Table BXII.γ.193. All species were studied in a single laboratory (the Enteric Reference Laboratories at the Centers for Disease Control and Prevention, Atlanta, GA, USA) by a single set of methods (Edwards and Ewing, 1972; Farmer et al., 1980a; Hickman and Farmer, 1978). Because different methods and tests are often used in different laboratories, the percentage of positive reactions obtained may differ somewhat from those presented in the chapters on specific genera. The purpose of Table BXII.γ.193 is not to advocate any given set of tests or to put undue emphasis on the percentages obtained, but to present a comprehensive comparison derived from a single set of data obtained by tests commonly done in a diagnostic laboratory. Commercial identification systems and computer-aided identification are also widely used. These methods can be supplemented by identification based on genus- and/or species-specific tests, which can be both sensitive and specific. (An example is bacteriophage O1, which is both sensitive and specific for the most common serotypes of *Salmonella*.) Several of these tests are listed in Table BXII.γ.194. Many genera and species of *Enterobacteriaceae* also have typical patterns of resistance and susceptibility to antibiotics; thus, the antibiogram of an isolate can also be used as an aid to identification (Table BXII.γ.195). Detailed discussions of practices in clinical microbiology laboratories for isolation, maintenance, storage, computer-aided identification, use of commercial test "kits", antibiotic susceptibility testing, and difficulties presented by "problem strains" of *Enterobacteriaceae* can be found in Brenner (1992b) and Farmer (1999).

From 50 to more than 200 biochemical tests have been used in phenetic or numerical taxonomic studies of *Enterobacteriaceae* (Bascomb et al., 1971; Johnson et al., 1975; Véron, 1975; Véron and Le Minor, 1975a, b). These include tests for the fermentation of a large number of carbohydrates and polyhydroxyl alcohols, tests for the ability to use a wide variety of organic substrates as the sole source of carbon and energy, and tests for the presence of specific enzymes. A number of these tests are useful for the differentiation of species, subspecies, or biogroups within *Enterobacteriaceae* (Véron and Le Minor, 1975a, b). Some tests of particular diagnostic value are for nitrate reductase type A or type B (Pichinoty and Piéchaud, 1968; Pichinoty et al., 1969), tetrathionate reductase (Richard, 1977), fermentation or growth on D-galacturonate (Le Minor et al., 1979), presence of α-glutamyl transferase (Giammanco et al., 1980), and fermentation or growth on 2-ketogluconate (Buissière et al., 1981). A summary of data obtained at the Institut Pasteur for these tests is given in Table BXII.γ.196. Other nonroutine tests of value in differentiating between phenotypically similar organisms are ascorbate and D-arabitol for *Buttiauxella* and *Kluyvera*; L-fucose, 5-ketogluconate, D-xylose, and D-sorbose to differentiate *Klebsiella* from *Enterobacter*; susceptibility to *Hafnia*-specific bacteriophage to differentiate *Hafnia* from other genera; pectinase to differentiate erwiniae from other genera; ascorbate, D-glucose fermentation at 5°C within 21 d, and irgasan susceptibility to differentiate *Kluyvera* species; tyrosine clearing to differentiate *Leminorella* species from biochemically similar species; growth in *trans*-aconitate, adonitol, benzoate, *m*-erythritol, gentisate, D-malate, L-rhamnose, and *m*-tartrate to differentiate *S. liquefaciens*, *S. proteamaculans*, and *S. grimesii*; growth in histamine, D-melizitose, and D-tartrate to differentiate *S. rubidaea* subspecies; and bioluminescence, pigment on Loeffler's blood serum, colony color on MacConkey agar, and adsorption of bromthymol blue to differentiate *Photorhabdus* and *Xenorhabdus* species. Reactions for species in these specialized tests are given in the following genus chapters.

Molecular methods can be used to identify bacteria at taxonomic levels from the family down to the strain; furthermore, molecular tests based on virulence and pathogenicity genes can be used to distinguish pathogenic and nonpathogenic isolates. Many of these methods have been described for *Enterobacteriaceae*. For example, detection of the *phoE* gene by PCR amplification provides a sensitive and specific test for *Escherichia* and *Shigella*. A growing number of genus or species-specific probes have been reported in the literature. A number of systems for identification by either complete or partial 16S rRNA gene sequencing are now under development.

TABLE BXII.γ.193. Biochemical reactions of the named species and unnamed Enteric Groups of the family *Enterobacteriaceae*[a, b]

Characteristic	*Escherichia coli*	*Escherichia coli*, inactive	*Escherichia blattae*	*Escherichia fergusonii*	*Escherichia hermannii*	*Escherichia vulneris*	*Budvicia aquatica*	*Buttiauxella agrestis*	*Buttiauxella brennerae*	*Buttiauxella ferragutiae*	*Buttiauxella gaviniae*	*Buttiauxella izardii*	*Buttiauxella noackiae*	*Buttiauxella warmboldiae*	*Cedecea davisae*	*Cedecea lapagei*
Indole production	98	80	0	98	99	0	0	0	0	0	0	0	33	0	0	0
Methyl red	99	95	100	100	100	100	93	100	100	100	100	100	100	100	100	40
Voges–Proskauer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	80
Citrate (Simmons)	1	1	50	17	1	0	0	100	0	0	20	0	33	33	95	99
Hydrogen sulfide (TSI)	1	1	0	0	0	0	80	0	0	0	0	0	0	0	0	0
Urea hydrolysis	1	1	0	0	0	0	33	0	0	0	0	0	0	0	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0
Lysine decarboxylase	90	40	100	95	6	85	0	0	0	100	0	0	0	0	0	0
Arginine dihydrolase	17	3	0	5	0	30	0	0	0	0	20	0	67	0	50	80
Ornithine decarboxylase	65	20	100	100	100	0	0	100	33	80	0	100	0	0	95	0
Motility	95	5	0	93	99	100	27	100	100	60	80	100	100	100	95	80
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	3	1	0	0	94	15	0	80	100	40	60	67	100	33	85	100
Malonate utilization	0	0	100	35	0	85	0	60	100	0	100	100	100	100	91	99
Esculin hydrolysis	35	5	0	46	40	20	0	100	100	100	100	100	100	100	45	100
Tartrate, Jordan's	95	85	50	96	35	2	27	60	0	0	40	67	100	0	0	0
Acetate utilization	90	40	0	96	78	30	0	0	0	0	0	0	0	0	0	60
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	100
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	95	45	0	83	98	100	93	100	100	100	100	100	100	100	90	99
Yellow pigment	0	0	0	0	98	50	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	95	5	100	95	97	97	53	100	100	100	40	100	100	100	70	100
Fermentation of:																
Adonitol	5	3	0	98	0	0	0	0	67	0	100	0	0	0	0	0
L-Arabinose	99	85	100	98	100	100	80	100	100	100	100	100	100	100	0	0
D-Arabitol	5	5	0	100	8	0	27	0	67	0	80	0	0	0	100	100
Cellobiose	2	2	0	96	97	100	0	100	100	100	100	100	100	100	100	100
Dulcitol	60	40	0	60	19	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	75	65	100	20	3	25	0	60	67	0	0	33	0	0	0	0
myo-Inositol	1	1	0	0	0	0	0	0	0	0	0	0	0	67	0	0
Lactose	95	25	0	0	45	15	87	100	0	0	60	100	0	0	19	60
Maltose	95	80	100	96	100	100	0	100	100	100	60	100	100	100	100	100
D-Mannitol	98	93	0	98	100	100	60	100	100	100	100	100	100	100	100	100
D-Mannose	98	97	100	100	100	100	0	100	100	100	100	100	100	100	100	100
Melibiose	75	40	0	0	0	100	0	100	100	0	0	67	0	0	0	0
α-Methyl-D-glucoside	0	0	0	0	0	25	0	0	0	40	0	0	33	0	5	0
Mucate	95	30	50	0	97	78	20	100	67	60	80	100	100	0	0	0
Raffinose	50	15	0	0	40	99	0	100	100	0	0	33	0	0	10	0
L-Rhamnose	80	65	100	92	97	93	100	100	33	100	100	100	100	100	0	0
Salicin	40	10	0	65	40	30	0	100	100	100	100	100	100	100	99	100
D-Sorbitol	94	75	0	0	0	1	0	0	0	100	0	0	0	0	0	0
Sucrose	50	15	0	0	45	8	0	0	0	0	0	0	0	0	100	0
Trehalose	98	90	75	96	100	100	0	100	100	100	100	100	100	100	100	100
D-Xylose	95	70	100	96	100	100	93	100	100	100	100	100	100	100	100	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Cedecea neteri*	*Cedecea* species 3	*Cedecea* species 5	*Citrobacter amalonaticus*	*Citrobacter freundii*	*Citrobacter braakii*	*Citrobacter farmeri*	*Citrobacter gillenii*	*Citrobacter koseri* (*C. diversus*)	*Citrobacter murliniae*	*Citrobacter rodentium*	*Citrobacter sedlakii*	*Citrobacter werkmanii*	*Citrobacter youngae*	*Edwardsiella tarda*	*Edwardsiella tarda* biogroup 1
Indole production	0	0	0	100	33	33	100	0	99	100	0	83	0	15	99	100
Methyl red	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Voges–Proskauer	50	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrate (Simmons)	100	100	100	95	78	87	10	33	99	100	0	83	100	75	1	0
Hydrogen sulfide (TSI)	0	0	0	5	78	60	0	67	0	67	0	0	100	65	100	0
Urea hydrolysis	0	0	0	85	44	47	59	0	75	67	100	100	100	80	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
Arginine dihydrolase	100	100	50	85	67	67	85	33	80	67	0	100	100	50	0	0
Ornithine decarboxylase	0	0	50	95	0	93	100	0	99	0	100	100	0	5	100	100
Motility	100	100	100	95	89	87	97	67	95	100	0	100	100	95	98	100
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	65	100	100	99	89	100	93	100	0	100	0	100	100	95	0	0
Malonate utilization	100	0	0	1	11	0	0	100	95	0	100	100	100	5	0	0
Esculin hydrolysis	100	100	100	5	0	0	0	0	1	0	0	17	0	5	0	0
Tartrate, Jordan's	0	0	0	96	100	93	93	100	90	100	100	100	100	100	25	0
Acetate utilization	0	50	50	86	44	53	80	0	75	33	0	83	100	65	0	0
Lipase (corn oil)	100	100	50	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	100	99	100	100	100	100	100	100	100	100	100	85	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	100	100	100	97	89	80	100	67	99	100	100	100	100	90	0	0
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	100	100	100	97	89	93	96	100	98	100	100	100	100	75	100	50
Fermentation of:																
Adonitol	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0
L-Arabinose	0	0	0	99	100	100	100	100	99	100	100	100	100	100	9	100
D-Arabitol	100	100	100	0	0	0	0	0	98	0	0	0	0	5	0	0
Cellobiose	100	100	100	100	44	73	100	67	99	100	100	100	0	45	0	0
Dulcitol	0	0	0	1	11	33	2	0	40	100	0	100	0	85	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	60	100	87	65	67	99	100	0	83	100	90	30	0
myo-Inositol	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Lactose	35	0	0	35	78	80	15	67	50	67	100	100	17	25	0	0
Maltose	100	100	100	99	100	100	100	100	100	100	100	100	100	95	100	100
D-Mannitol	100	100	100	100	100	100	100	100	99	100	100	100	100	100	0	100
D-Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Melibiose	0	100	100	0	100	80	100	67	0	33	0	100	0	10	0	0
α-Methyl-D-glucoside	0	50	0	2	11	33	75	0	40	0	0	0	0	0	0	0
Mucate	0	0	0	96	100	100	100	67	95	100	100	100	100	100	0	0
Raffinose	0	100	100	5	44	7	100	0	0	33	0	0	0	10	0	0
L-Rhamnose	0	0	0	100	100	100	100	100	99	100	100	100	100	100	0	0
Salicin	100	100	100	30	0	0	9	0	15	33	0	17	0	10	0	0
D-Sorbitol	100	0	100	99	100	100	98	100	99	100	100	100	100	100	0	0
Sucrose	100	50	100	9	89	7	100	33	40	33	0	0	0	20	0	100
Trehalose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
D-Xylose	100	100	100	99	89	100	100	100	100	100	100	100	100	100	0	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Edwardsiella hoshinae*	*Edwardsiella ictaluri*	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Enterobacter amnigenus* biogroup 1	*Enterobacter amnigenus* biogroup 2	*Enterobacter asburiae*	*Enterobacter cancerogenus* (*E. taylorae*)	*Enterobacter dissolvens*	*Enterobacter gergoviae*	*Enterobacter hormaechei*	*Enterobacter intermedius*	*Enterobacter nimipressuralis*	*Enterobacter pyrinus*	*Enterobacter sakazakii*	*Ewingella americana*
Indole production	50	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
Methyl red	100	0	5	5	7	65	100	5	0	5	57	100	100	29	5	84
Voges–Proskauer	0	0	100	98	100	100	2	100	100	100	100	100	100	86	100	95
Citrate (Simmons)	0	0	100	95	70	100	100	100	100	99	96	65	0	0	99	95
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea hydrolysis	0	0	65	2	0	0	60	1	100	93	87	0	0	86	1	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	4	0	0	0	50	0
Lysine decarboxylase	100	100	0	98	0	0	0	0	0	90	0	0	0	100	0	0
Arginine dihydrolase	0	0	97	0	9	35	21	94	100	0	78	0	0	0	99	0
Ornithine decarboxylase	95	65	96	98	55	100	95	99	100	100	91	89	100	100	91	0
Motility	100	0	95	97	92	100	0	99	0	90	52	89	0	43	96	60
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	0	0	98	98	100	100	97	98	100	0	100	65	100	0	99	5
Malonate utilization	100	0	75	95	91	100	3	100	100	96	100	100	100	86	18	0
Esculin hydrolysis	0	0	30	98	91	100	95	90	100	97	0	100	100	100	100	50
Tartrate, Jordan's	0	0	30	95	9	0	30	0	0	97	13	100	0	0	1	35
Acetate utilization	0	0	75	50	0	0	87	35	100	93	74	0	0	0	96	10
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	99	100	100	100	100	100	100	99	100	100	100	100	99	97
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	0	0	99	100	91	100	100	100	100	97	95	100	100	100	100	85
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	35	50	100	100	100	100	95	100	100	98	83	100	100	100	98	0
Fermentation of:																
Adonitol	0	0	25	98	100	0	0	0	0	0	0	0	0	0	0	0
L-Arabinose	13	0	100	100	100	100	100	100	100	99	100	100	100	100	100	0
D-Arabitol	0	0	15	100	0	0	0	0	0	97	0	0	0	0	0	99
Cellobiose	0	0	99	100	100	100	100	100	100	99	100	100	100	100	100	10
Dulcitol	0	0	15	5	0	0	0	0	0	0	87	100	0	0	5	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	65	0	40	98	0	0	11	1	0	100	4	100	0	0	15	24
myo-Inositol	0	0	15	95	100	0	0	0	0	0	0	0	0	100	75	0
Lactose	0	0	93	95	70	35	75	10	0	55	9	100	0	14	99	70
Maltose	100	100	100	99	100	100	100	99	100	100	100	100	100	100	100	16
D-Mannitol	100	0	100	100	100	100	100	100	100	99	100	100	100	100	100	100
D-Mannose	100	100	100	95	100	100	100	100	100	100	100	100	100	100	100	99
Melibiose	0	0	90	99	100	100	0	0	100	97	0	100	100	0	100	0
α-Methyl-D-glucoside	0	0	85	95	55	100	95	1	100	2	83	100	100	0	96	0
Mucate	0	0	75	90	35	100	21	75	100	2	96	100	100	0	1	0
Raffinose	0	0	97	96	100	0	70	0	100	97	0	100	0	0	99	0
L-Rhamnose	0	0	92	99	100	100	5	100	100	99	100	100	100	100	100	23
Salicin	50	0	75	100	91	100	100	92	100	99	44	100	100	100	99	80
D-Sorbitol	0	0	95	100	9	100	100	1	100	0	0	100	100	0	0	0
Sucrose	100	0	97	100	100	0	100	0	100	98	100	65	0	100	100	0
Trehalose	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	99
D-Xylose	0	0	99	100	100	100	97	100	100	99	96	100	100	0	100	13

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Hafnia alvei*	*Hafnia alvei* biogroup 1	*Klebsiella pneumoniae* subsp. *ozaenae*	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	*Klebsiella oxytoca*	*Klebsiella oxytoca*, ornithine positive	*Klebsiella planticola*	*Klebsiella terrigena*	*Kluyvera ascorbata*	*Kluyvera cryocrescens*	*Kluyvera georgiana*	*Leclercia adecarboxylata*	*Leminorella grimontii*	*Leminorella richardii*	*Moellerella wisconsensis*
Indole production	0	0	0	0	0	99	100	20	0	92	90	100	100	0	0	0
Methyl red	40	85	98	10	100	20	96	100	60	100	100	100	100	100	0	100
Voges–Proskauer	85	70	0	98	0	95	70	98	100	0	0	0	0	0	0	0
Citrate (Simmons)	10	0	30	98	0	95	100	100	40	96	80	100	0	100	0	80
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0
Urea hydrolysis	4	0	10	95	0	90	100	98	0	0	0	0	48	0	0	0
Phenylalanine deaminase	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	100	100	40	98	0	99	100	100	100	97	23	100	0	0	0	0
Arginine dihydrolase	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase	98	45	3	0	0	0	100	0	20	100	100	100	0	0	0	0
Motility	85	0	0	0	0	0	0	0	0	98	90	100	79	0	0	0
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	95	0	88	98	80	97	100	100	100	92	86	83	97	0	0	70
Malonate utilization	50	45	3	93	95	98	100	100	100	96	86	50	93	0	0	0
Esculin hydrolysis	7	0	80	99	30	100	100	100	100	99	100	100	100	0	0	0
Tartrate, Jordan's	70	30	50	95	50	98	100	100	100	35	19	50	83	100	100	30
Acetate utilization	15	0	2	75	0	90	95	62	20	50	86	83	28	0	0	10
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	80	99	100	100	100	100	100	100	100	100	100	100	100	90
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	90	30	80	99	0	100	100	100	100	100	100	100	100	0	0	90
Yellow pigment	0	0	0	0	0	1	0	1	0	0	0	0	37	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	98	0	50	97	0	97	100	100	80	93	95	17	97	33	0	0
Fermentation of:																
Adonitol	0	0	97	90	100	99	100	100	100	0	0	0	93	0	0	100
L-Arabinose	95	0	98	99	100	98	100	100	100	100	100	100	100	100	100	0
D-Arabitol	0	0	95	98	100	98	100	100	100	0	0	0	96	0	0	75
Cellobiose	15	0	92	98	100	100	100	100	100	100	100	100	100	0	0	0
Dulcitol	0	0	2	30	0	55	10	15	20	25	0	33	86	83	0	0
Erythritol	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Glycerol	95	0	65	97	50	99	100	100	100	40	5	33	3	17	0	10
myo-Inositol	0	0	55	95	95	98	95	100	80	0	0	0	0	0	0	0
Lactose	5	0	30	98	0	100	100	100	100	98	95	83	93	0	0	100
Maltose	100	0	95	98	100	100	100	100	100	100	100	100	100	0	0	30
D-Mannitol	99	55	100	99	100	99	100	100	100	100	95	100	100	0	0	60
D-Mannose	100	100	100	99	100	100	100	100	100	100	100	100	100	0	0	100
Melibiose	0	0	97	99	100	99	100	100	100	99	100	100	100	0	0	100
α-Methyl-D-glucoside	0	0	70	90	0	98	100	100	100	98	95	100	0	0	0	0
Mucate	0	0	25	90	0	93	96	100	100	90	81	83	93	100	50	0
Raffinose	2	0	90	99	90	100	100	100	100	98	100	100	66	0	0	100
L-Rhamnose	97	0	55	99	96	100	100	100	100	100	100	83	100	0	0	0
Salicin	13	55	97	99	98	100	100	100	100	100	100	100	100	0	0	0
D-Sorbitol	0	0	65	99	100	99	100	92	100	40	45	0	0	0	0	0
Sucrose	10	0	20	99	75	100	100	100	100	98	81	100	66	0	0	100
Trehalose	95	70	98	99	100	100	100	100	100	100	100	100	100	0	0	0
D-Xylose	98	0	95	99	100	100	100	100	100	99	91	100	100	83	100	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Morganella morganii* subsp. *morganii*	*Morganella morganii* subsp. *sibonii*	*Morganella morganii* biogroup 1	*Obesumbacterium proteus* biogroup 2	*Pantoea agglomerans*	*Pantoea dispersa*	*Photorhabdus luminescens* (all tests at 25°C)	*Photorhabdus* DNA hybridization group 5	*Pragia fontium*	*Proteus vulgaris*	*Proteus mirabilis*	*Proteus myxofaciens*	*Proteus penneri*	*Providencia alcalifaciens*	*Providencia heimbachae*	*Providencia rettgeri*
Indole production	95	50	100	0	20	0	50	0	0	98	2	0	0	99	0	99
Methyl red	95	86	95	15	50	82	0	0	100	95	97	100	100	99	85	93
Voges–Proskauer	0	0	0	0	70	64	0	0	0	0	50	100	0	0	0	0
Citrate (Simmons)	0	0	0	0	50	100	50	20	89	15	65	50	0	98	0	95
Hydrogen sulfide (TSI)	20	7	15	0	0	0	0	0	89	95	98	0	30	0	0	0
Urea hydrolysis	95	100	100	0	20	0	25	60	0	95	98	100	100	0	0	98
Phenylalanine deaminase	95	93	100	0	20	9	0	0	22	99	98	100	99	98	100	98
Lysine decarboxylase	1	29	100	100	0	0	0	0	0	0	0	0	0	0	0	0
Arginine dihydrolase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase	95	64	80	100	0	0	0	0	0	0	99	0	0	1	0	0
Motility	95	79	0	0	85	100	100	100	100	95	95	100	85	96	46	94
Gelatin hydrolysis (22°C)	0	0	0	0	2	0	50	80	0	91	90	100	50	0	0	0
Growth in KCN	98	79	90	0	35	82	0	20	0	99	98	100	99	100	8	97
Malonate utilization	1	0	5	0	65	9	0	0	0	0	2	0	0	0	0	0
Esculin hydrolysis	0	0	0	0	60	0	0	0	78	50	0	0	0	0	0	35
Tartrate, Jordan's	95	100	100	15	25	9	50	60	0	80	87	100	85	90	69	95
Acetate utilization	0	0	0	0	30	100	0	20	0	25	20	0	5	40	0	60
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	80	92	100	45	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	80	50	50	40	0	0	0
Nitrate oxidized to nitrite	90	100	90	100	85	91	0	0	100	98	95	100	90	100	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	10	0	20	0	90	91	0	0	0	1	0	0	1	1	0	5
Yellow pigment	0	0	0	0	75	27	50	60	0	0	0	0	0	0	0	0
D-Glucose, acid	99	100	100	100	100	100	75	100	100	100	100	100	100	100	100	100
D-Glucose, gas	90	86	93	0	20	0	0	0	0	85	96	100	45	85	0	10
Fermentation of:																
Adonitol	0	0	0	0	7	0	0	0	0	0	0	0	0	98	92	100
L-Arabinose	0	0	0	0	95	100	0	0	0	0	0	0	0	1	0	0
D-Arabitol	0	0	0	0	50	100	0	0	0	0	0	0	0	0	92	100
Cellobiose	0	0	0	0	55	55	0	0	0	0	1	0	0	0	0	3
Dulcitol	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	75
Glycerol	5	7	100	0	30	27	0	0	0	60	70	100	55	15	0	60
myo-Inositol	0	0	0	0	15	0	0	0	0	0	0	0	0	1	46	90
Lactose	1	0	0	0	40	0	0	0	0	2	2	0	1	0	0	5
Maltose	0	0	0	50	89	82	25	0	0	97	0	100	100	1	54	2
D-Mannitol	0	0	0	0	100	100	0	0	0	0	0	0	0	2	0	100
D-Mannose	98	100	100	85	98	100	100	100	0	0	0	0	0	100	100	100
Melibiose	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	5
α-Methyl-D-glucoside	0	0	0	0	7	0	0	0	0	60	0	100	80	0	0	2
Mucate	0	7	0	0	40	0	0	0	0	0	0	0	0	0	0	0
Raffinose	0	0	0	0	30	0	0	0	0	1	1	0	1	1	0	5
L-Rhamnose	0	0	0	15	85	91	0	0	0	5	1	0	0	0	100	70
Salicin	0	0	0	0	65	0	0	0	78	50	0	0	0	1	0	50
D-Sorbitol	0	0	0	0	30	0	0	0	0	0	0	0	0	1	0	1
Sucrose	0	7	0	0	75	1	0	0	0	97	15	100	100	15	0	15
Trehalose	0	100	0	85	97	100	0	0	0	30	98	100	55	2	0	0
D-Xylose	0	0	0	15	93	100	0	0	0	95	98	0	100	1	8	10

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Providencia rustigianii*	*Providencia stuartii*	*Rahnella aquatilis*	*Salmonella bongori*	*Salmonella enterica* subsp. *enterica*[c]	*Salmonella enterica* subsp. *arizonae*[c]	*Salmonella enterica* subsp. *diarizonae*[c]	*Salmonella enterica* subsp. *houtenae*[c]	*Salmonella enterica* subsp. *indica*[c]	*Salmonella enterica* subsp. *salamae*[c]	*Salmonella* serovar *Choleraesuis*[c]	*Salmonella* serovar *Gallinarum*[c]	*Salmonella* serovar *Paratyphi A*[c]	*Salmonella* serovar *Pullorum*[c]	*Salmonella* serovar *Typhi*[c]	*Serratia marcescens*
Indole production	98	98	0	0	1	1	2	0	0	2	0	0	0	0	0	1
Methyl red	65	100	88	100	100	100	100	100	100	100	100	100	100	90	100	20
Voges–Proskauer	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	98
Citrate (Simmons)	15	93	94	94	95	99	98	98	89	100	25	0	0	0	0	98
Hydrogen sulfide (TSI)	0	0	0	100	95	99	99	100	100	100	50	100	10	90	97	0
Urea hydrolysis	0	30	0	0	1	0	0	2	0	0	0	0	0	0	0	15
Phenylalanine deaminase	100	95	95	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	0	0	0	100	98	99	99	100	100	100	95	90	0	100	98	99
Arginine dihydrolase	0	0	0	94	70	70	70	70	67	90	55	10	15	10	3	0
Ornithine decarboxylase	0	0	0	100	97	99	99	100	100	100	100	1	95	95	0	99
Motility	30	85	6	100	95	99	99	98	100	98	95	0	95	0	97	97
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	90
Growth in KCN	100	100	0	100	0	1	1	95	0	0	0	0	0	0	0	95
Malonate utilization	0	0	100	0	0	95	95	0	0	95	0	0	0	0	0	3
Esculin hydrolysis	0	0	100	0	5	1	1	0	0	15	0	0	0	0	0	95
Tartrate, Jordan's	50	90	6	0	90	5	20	65	100	50	85	100	0	0	100	75
Acetate utilization	25	75	6	100	90	90	75	70	89	95	1	0	0	0	0	50
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98
DNase (25°C)	0	10	0	0	2	2	2	0	0	0	0	10	0	0	0	98
Nitrate oxidized to nitrite	100	100	100	100	100	100	100	100	100	100	98	100	100	100	100	98
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	0	10	100	94	2	100	92	0	44	15	0	0	0	0	0	95
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	35	0	98	94	96	99	99	100	100	100	95	0	99	90	0	55
Fermentation of:																
Adonitol	0	5	0	0	0	0	0	5	0	0	0	0	0	0	0	40
L-Arabinose	0	1	100	94	99	99	99	100	100	100	0	80	100	100	2	0
D-Arabitol	0	0	0	0	0	1	1	5	0	0	1	0	0	0	0	0
Cellobiose	0	5	100	0	5	1	1	50	0	0	0	10	5	5	0	5
Dulcitol	0	0	88	94	96	0	1	0	67	90	5	90	90	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1
Glycerol	5	50	13	0	5	10	10	0	33	25	0	0	10	0	20	95
myo-Inositol	0	95	0	0	35	0	0	0	0	5	0	0	0	0	0	75
Lactose	0	2	100	0	1	15	85	0	22	1	0	0	0	0	1	2
Maltose	0	1	94	100	97	98	98	100	100	100	95	90	95	5	97	96
D-Mannitol	0	10	100	100	100	100	100	98	100	100	98	100	100	100	100	99
D-Mannose	100	100	100	100	100	100	100	100	100	95	95	100	100	100	100	99
Melibiose	0	0	100	94	95	95	95	100	89	8	45	0	95	0	100	0
α-Methyl-D-glucoside	0	0	0	0	2	1	1	0	0	8	0	0	0	0	0	0
Mucate	0	0	30	88	90	90	30	0	89	96	0	50	0	0	0	0
Raffinose	0	7	94	0	2	1	1	0	0	0	1	10	0	1	0	2
L-Rhamnose	0	0	94	88	95	99	99	98	100	100	100	10	100	100	0	0
Salicin	0	2	100	0	0	0	0	60	0	5	0	0	0	0	0	95
D-Sorbitol	0	1	94	100	95	99	99	100	0	100	90	1	95	10	99	99
Sucrose	35	50	100	0	1	1	5	0	0	1	0	0	0	0	0	99
Trehalose	0	98	100	100	99	99	99	100	100	100	0	50	100	90	100	99
D-Xylose	0	7	94	100	97	100	100	100	100	100	98	70	0	90	82	7

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Serratia entomophila*	*Serratia ficaria*	*Serratia fonticola*	*Serratia liquefaciens*	*Serratia marcescens* biogroup 1	*Serratia odorifera* biogroup 1	*Serratia odorifera* biogroup 2	*Serratia plymuthica*	*Serratia rubidaea*	*Shigella dysenteriae*	*Shigella boydii*	*Shigella flexneri*	*Shigella sonnei*	*Tatumella ptyseos*	*Trabulsiella guamensis*	*Xenorhabdus nematophilus*
Indole production	0	0	0	1	0	60	50	0	0	45	25	50	0	0	40	40
Methyl red	20	75	100	93	100	100	60	94	20	99	100	100	100	0	100	0
Voges–Proskauer	100	75	9	93	60	50	100	80	100	0	0	0	0	5	0	0
Citrate (Simmons)	100	100	91	90	30	100	97	75	95	0	0	0	0	2	88	0
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Urea hydrolysis	0	0	13	3	0	5	0	0	2	0	0	0	0	0	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	0	90	0	0
Lysine decarboxylase	0	0	100	95	55	100	94	0	55	0	0	0	0	0	100	0
Arginine dihydrolase	0	0	0	0	4	0	0	0	0	2	18	5	2	0	50	0
Ornithine decarboxylase	0	0	97	95	65	100	0	0	0	0	2	0	98	0	100	0
Motility	100	100	91	95	17	100	100	50	85	0	0	0	0	0	100	100
Gelatin hydrolysis (22°C)	100	100	0	90	30	95	94	60	90	0	0	0	0	0	0	80
Growth in KCN	100	55	70	90	70	60	19	30	25	0	0	100	0	0	100	0
Malonate utilization	0	0	88	2	0	0	0	0	94	100	0	0	0	0	0	0
Esculin hydrolysis	100	100	100	97	96	95	40	81	94	0	0	0	0	0	40	0
Tartrate, Jordan's	100	17	58	75	50	100	100	100	70	75	50	30	90	0	50	60
Acetate utilization	80	40	15	40	4	60	65	55	80	0	0	8	0	0	88	0
Lipase (corn oil)	20	77	0	85	75	35	65	70	99	0	0	0	0	0	0	0
DNase (25°C)	100	100	0	85	82	100	100	100	99	0	0	0	0	0	0	20
Nitrate oxidized to nitrite	100	92	100	100	83	100	100	100	100	99	100	99	100	98	100	20
Oxidase, Kovacs	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	100	100	100	93	75	100	100	70	100	30	10	1	90	0	100	0
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60
D-Glucose, acid	100	100	100	100	100	100	100	100	100	0	100	100	100	100	100	80
D-Glucose, gas	0	0	79	75	0	0	13	40	30	0	0	3	0	0	100	0
Fermentation of:																
Adonitol	0	0	100	5	30	50	55	0	99	0	0	0	0	0	0	0
L-Arabinose	0	100	100	98	0	100	100	100	100	45	94	60	95	0	100	0
D-Arabitol	60	100	100	0	0	0	0	0	85	0	0	1	0	0	0	0
Cellobiose	0	100	6	5	4	100	100	88	94	0	0	0	5	0	100	0
Dulcitol	0	0	91	0	0	0	0	0	0	0	5	1	0	0	0	0
Erythritol	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
Glycerol	0	0	88	95	92	40	50	50	20	10	50	10	15	7	0	0
myo-Inositol	0	55	30	60	30	100	100	50	20	0	0	0	0	0	0	0
Lactose	0	15	97	10	4	70	97	80	100	0	1	1	2	0	0	0
Maltose	100	100	97	98	70	100	100	94	99	15	20	30	90	0	100	0
D-Mannitol	100	100	100	100	96	100	97	100	100	100	97	95	99	0	100	0
D-Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	80
Melibiose	0	40	98	75	0	100	96	93	99	0	15	55	25	25	0	0
α-Methyl-D-glucoside	0	8	91	5	0	0	0	70	1	0	0	0	0	0	0	0
Mucate	0	0	0	0	0	5	0	0	0	0	0	0	10	0	100	0
Raffinose	0	70	100	85	0	100	7	94	99	0	0	40	3	11	0	0
L-Rhamnose	0	35	76	15	0	95	94	0	1	30	1	5	75	0	100	0
Salicin	100	100	100	97	92	98	45	94	99	0	0	0	0	55	13	0
D-Sorbitol	0	100	100	95	92	100	100	65	1	30	43	29	2	0	100	0
Sucrose	100	100	21	98	100	100	0	100	99	1	0	1	1	98	0	0
Trehalose	100	100	100	100	100	100	100	100	100	90	85	65	100	93	100	0
D-Xylose	40	100	85	100	0	100	100	94	99	4	11	2	2	9	100	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Yersinia pestis*	*Yersinia aldovae*	*Yersinia bercovieri*	*Yersinia enterocolitica*	*Yersinia frederiksenii*	*Yersinia intermedia*	*Yersinia kristensenii*	*Yersinia mollaretii*	*Yersinia pseudotuberculosis*	*Yersinia rohdei*	*Yersinia ruckeri*	*Yokenella regensburgei* (*Koserella trabulsii*)	Enteric Group 58	Enteric Group 60	Enteric Group 68	Enteric Group 69
Indole production	0	0	0	50	100	100	30	0	0	0	0	0	0	0	0	0
Methyl red	80	80	100	97	100	100	92	100	100	62	97	100	100	100	100	0
Voges–Proskauer	0	0	0	2	0	5	0	0	0	0	10	0	0	0	50	100
Citrate (Simmons)	0	0	0	0	15	5	0	0	0	0	0	92	85	0	0	100
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea hydrolysis	5	60	60	75	70	80	77	20	95	62	0	0	70	50	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	0	0	0	0	0	0	0	0	0	0	50	100	100	0	0	0
Arginine dihydrolase	0	0	0	0	0	0	0	0	0	0	5	8	0	0	0	100
Ornithine decarboxylase	0	40	80	95	95	100	92	80	0	25	100	100	85	100	0	100
Motility	0	0	0	2	5	5	5	0	0	0	0	100	100	75	0	100
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0
Growth in KCN	0	0	0	2	0	10	0	0	0	0	15	92	100	0	100	100
Malonate utilization	0	0	0	0	0	5	0	0	0	0	0	0	85	100	0	100
Esculin hydrolysis	50	0	20	25	85	100	0	0	95	0	0	67	0	0	0	100
Tartrate, Jordan's	0	100	100	85	55	88	40	100	50	100	30	0	60	75	0	0
Acetate utilization	0	0	0	15	15	18	8	0	0	0	0	25	45	0	0	25
Lipase (corn oil)	0	0	0	55	55	12	0	0	0	0	30	0	0	0	0	0
DNase (25°C)	0	0	0	5	0	0	0	0	0	0	0	0	0	0	100	0
Nitrate oxidized to nitrite	85	100	100	98	100	94	100	100	95	88	75	100	100	100	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	50	0	80	95	100	90	70	20	70	50	50	100	100	100	0	100
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	0	0	0	5	40	18	23	0	0	0	5	100	85	100	0	100
Fermentation of:																
Adonitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Arabinose	100	60	100	98	100	100	77	100	50	100	5	100	100	25	0	100
D-Arabitol	0	0	0	40	100	45	45	0	0	0	0	0	0	0	0	0
Cellobiose	0	0	100	75	100	96	100	100	0	25	5	100	100	0	0	100
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	85	0	0	100
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	50	0	0	90	85	60	70	20	50	38	30	0	30	75	50	0
myo-Inositol	0	0	0	30	20	15	15	0	0	0	0	0	0	0	0	0
Lactose	0	0	20	5	40	35	8	40	0	0	0	0	30	0	0	100
Maltose	80	0	100	75	100	100	100	60	95	0	95	100	100	0	50	100
D-Mannitol	97	80	100	98	100	100	100	100	100	100	100	100	100	50	100	100
D-Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Melibiose	20	0	0	1	0	80	0	0	70	50	0	92	0	0	0	100
α-Methyl-D-glucoside	0	0	0	0	0	77	0	0	0	0	0	0	55	0	0	100
Mucate	0	0	0	0	5	6	0	0	0	0	0	0	0	0	0	100
Raffinose	0	0	0	5	30	45	0	0	15	62	5	25	0	0	0	100
L-Rhamnose	1	0	0	1	99	100	0	0	70	0	0	100	100	75	0	100
Salicin	70	0	20	20	92	100	15	20	25	0	0	8	100	0	50	100
D-Sorbitol	50	60	100	99	100	100	100	100	0	100	50	0	100	0	0	100
Sucrose	0	20	100	95	100	100	0	100	0	100	0	0	0	0	100	25
Trehalose	100	80	100	98	100	100	100	100	100	100	95	100	100	100	100	100
D-Xylose	90	40	100	70	100	100	85	60	100	38	0	100	100	0	0	100

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

TABLE BXII.γ.194. Screening tests for genera and species of *Enterobacteriaceae* often isolated from human clinical specimens[a]

Organism (genus, species, or serovar)	Test or property[b, c, d]
Salmonella	Lactose$^-$, sucrose$^-$, H_2S^+, 01 phage$^+$[e], MUCAP$^+$, agglutinates in polyvalent serum[d], typical colonies on media selective/differential for *Salmonella* (brilliant green agar, SS agar, Rambach agar, etc.), lysed by the *Salmonella* specific bacteriophage[e], often antibiotic resistant
Salmonella Typhi	H_2S^+ (trace amount only), agglutinates in group D serum
Shigella	Nonmotile, lysine$^-$, gas$^-$, agglutinates in polyvalent serum, biochemically inactive, often antibiotic resistant, molecular test: *phoE*$^+$
Shigella dysenteriae	Agglutinates in group A serum, D-mannitol$^-$
Shigella dysenteriae O1	Catalase$^-$, agglutinates in O1 serum, Shiga toxin$^+$
Shigella flexneri	Agglutinates in group B serum, D-mannitol$^+$
Shigella boydii	Agglutinates in group C serum, D-mannitol$^+$
Shigella sonnei	Agglutinates in group D serum, D-mannitol$^+$
Escherichia coli	Extremely variable biochemically, indole$^+$, MUG$^+$, grows at 44.5°C, sometimes antibiotic resistant, molecular test: *phoE*$^+$
Escherichia coli O157:H7	Colorless colonies on sorbitol-MacConkey agar, MUG$^-$, D-sorbitol$^-$ (or delayed), agglutinates in O157 serum and H7 serum
Yersinia	Grows on CIN agar; often more active biochemically at 25°C than 36°C; motile at 25°C, nonmotile at 36°C; urea$^+$
Yersinia enterocolitica (pathogenic serogroups)	CR-MOX$^+$, pyrazinamidase$^-$, salicin$^-$, esculin$^-$, agglutinates in O sera: 3; 4, 32; 5, 27; 8; 9; 13a, 13b; 18; 20; or 21
Yersinia enterocolitica O3 (a pathogenic serogroup)	D-Xylose$^-$, agglutinates in O3 serum, tiny colonies at 24 h on plating media
Yersinia enterocolitica nonpathogenic serogroups	CR-MOX$^-$, pyrazinamidase$^+$, salicin$^+$, esculin$^+$, no agglutination in O sera: 3; 4, 32; 5, 27; 8; 9;13a, 13b; 18; 20 or 21
Citrobacter	Citrate$^+$, lysine decarboxylase$^-$, often grows on CIN agar, strong characteristic odor
Hafnia	Lysed by *Hafnia*-specific bacteriophage[e], often more active biochemically at 25°C than 36°C
Klebsiella	Mucoid colonies, encapsulated cells, nonmotile, lysine$^+$, very active biochemically, ferments most sugars, VP$^+$, malonate$^+$, resistant to carbenicillin and ampicillin
Enterobacter	Variable biochemically, citrate$^+$, VP$^+$, resistant to cephalothin
Serratia	DNase$^+$, gelatinase$^+$, lipase$^+$, resistant to colistin and cephalothin
Serratia marcescens	L-arabinose$^-$
Serratia, other species	L-arabinose$^+$
Proteus-Providencia-Morganella	Phenylalanine$^+$, tyrosine hydrolysis$^+$, often urea$^+$, resistant to colistin
Proteus	Swarms on blood agar, pungent odor, H_2S^+, gelatin$^+$, lipase$^+$
Proteus mirabilis	Urea$^+$, indole$^-$, ornithine decarboxylase$^+$, maltose$^-$
Proteus vulgaris	Urea$^+$, indole$^+$, ornithine decarboxylase$^-$, maltose$^+$
Providencia	No swarming, H_2S^-, ornithine decarboxylase$^-$, lipase$^-$
Morganella	Very inactive biochemically, no swarming, citrate$^-$, H_2S^-, ornithine decarboxylase$^+$, gelatin$^-$, lipase$^-$, urea$^+$

[a]Table taken from Farmer, 1999.

[b]Abbreviations: CIN, cefsulodin-irgasan-novobiocin agar (a plating medium selective for *Yersinia*); CR-MOX, Congo red, magnesium oxalate agar (a differential medium useful for distinguishing pathogenic from nonpathogenic strains of *Yersinia*); MUCAP, 4-methyl-umbelliferyl caprylate (a genus-specific test for *Salmonella*); MUG, 4-methyl-umbelliferyl-β-D-glucuronidase; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; *phoE*, a test done by PCR that is sensitive and specific for *E. coli*/*Shigella*; VP, Voges–Proskauer.

[c]This table gives only the general properties of the genera, species, and serogroups; exceptions occur. See Table BXII.γ.193 for full biochemical reactions. The properties listed for a genus or group of genera generally apply for each of its species, and the properties listed for a species generally apply for each of its serogroups.

[d]Biochemical test results are given as percentages in Table BXII.γ.193. The serological tests refer to slide agglutination in group or individual antisera (O1, O3, etc.) for *Salmonella*, *Shigella*, *E. coli*, or *Yersinia*, respectively.

[e]These are bacteriophage tests useful for identification.

Taxonomy and Nomenclature Compared with the 1st edition of *Bergey's Manual of Systematic Bacteriology* and the 9th edition of *Bergey's Manual of Determinative Bacteriology*, the present volume contains nomenclatural changes, new genera, and new species. In Table BXII.γ.192, the authors' proposed classification is compared with that in the previous two *Manuals*. Nomenclatural synonyms exist for a number of species, as shown in Table BXII.γ.192.

Arsenophonus A second species has provisionally been added to the genus as *Arsenophonus triatominarum*. The *candidatus* status was used because this species has only been recognized on the basis of its 16S rDNA sequence, and has not been isolated or characterized biochemically (Hypsa and Dale, 1997).

***Candidatus* Phlomobacter fragariae** The designation "Candidatus" is given to presumed new species that have not been cultivated. One such organism has been described whose 16S rDNA sequences place it within the family *Enterobacteriaceae* (Zreik et al., 1998). It has been named, *Candidatus* Phlomobacter fragariae. It is restricted to the phloem of strawberries and is associated with marginal chlorosis of strawberry (Zreik et al., 1998).

Citrobacter *Citrobacter koseri*, *Citrobacter diversus*, and *Levinea malonatica* are all synonyms for the same species. In response to a request for an opinion (Frederiksen, 1990), the Judicial Commission issued the opinion that *C. diversus* was a *nomina rejicienda* (rejected name). Therefore, the correct name is *C. koseri*. Eight new *Citrobacter* species were recently described (Brenner et al., 1993; Schauer et al., 1995). *Citrobacter farmeri* was formerly known as a biogroup of *C. amalonaticus*. The remaining 6 named and 2 unnamed species were previously included in *C. freundii* or had been previously identified as atypical citrobacteria.

Enterobacter *Enterobacter cancerogenus* (formerly *Erwinia cancerogena*) was shown to be a senior subjective synonym of *Enterobacter taylorae* and is therefore the correct name for this species (Grimont and Ageron, 1989). In several recently proposed classifi-

TABLE BXII.γ.195. Intrinsic antimicrobial resistance in some of the common *Enterobacteriaceae*[a]

Genus/Species	Most strains are resistant to
Buttiauxella species	Cephalothin
Cedecea species	Polymyxins, ampicillin, cephalothin
Citrobacter amalonaticus	Ampicillin
Citrobacter freundii	Cephalothin
Citrobacter diversus	Cephalothin, carbenicillin
Edwardsiella tarda	Colistin
Enterobacter cloacae	Cephalothin
Enterobacter aerogenes	Cephalothin
Many other *Enterobacter* species	Cephalothin
Escherichia hermannii	Ampicillin, carbenicillin
Ewingella americana	Cephalothin
Hafnia alvei	Cephalothin
Klebsiella pneumoniae	Ampicillin, carbenicillin
Kluyvera ascorbata	Ampicillin
Kluyvera cryocrescens	Ampicillin
Proteus mirabilis	Polymyxins, tetracycline, nitrofurantoin
Proteus vulgaris	Polymyxins, ampicillin, nitrofurantoin, tetracycline
Morganella morganii	Polymyxins, ampicillin, cephalothin
Providencia rettgeri	Polymyxins, cephalothin, nitrofurantoin, tetracycline
Other *Providencia* species[b]	Polymyxins, nitrofurantoin
Serratia marcescens[c]	Polymyxins, cephalothin, nitrofurantoin
Serratia fonticola	Ampicillin, carbenicillin, cephalothin
Other *Serratia* species	Polymyxins[d], cephalothin

[a]Table taken from Farmer, 1999.

[b]Most strains of *Providencia stuartii* are also resistant to cephalothin and tetracycline.

[c]*Serratia marcescens* can also be resistant to ampicillin, carbenicillin, streptomycin, and tetracycline.

[d]Resistance to polymyxins is common in *Serratia* species, but some strains have zones of 10–12 mm or larger.

cations, *Enterobacter agglomerans* and other species in the "*E. agglomerans* group" have been classified in the genus *Pantoea*. The *E. agglomerans* group was extremely heterogenous and a number of species have been proposed for strains previously included in this species (see discussions of *Erwinia*, *Leclercia*, and *Pantoea* below and Table BXII.γ.192). *E. aerogenes* is genetically and phenotypically closer to *K. pneumoniae* than to *Enterobacter cloacae* (Brenner et al., 1972c; Steigerwalt et al., 1976; Bascomb et al., 1971; Grimont and Grimont, 1992). The names *"Klebsiella aerogenes"* and *Klebsiella mobilis* have been used for *E. aerogenes*. The type strain of *E. cloacae* is not representative of the species. If it is maintained, typical strains identified in clinical laboratories might not be called *E. cloacae* (Grimont and Grimont, 1992). This problem is dealt with in detail in the chapter on *Enterobacter*. Recently obtained biochemical test data and DNA–DNA hybridization data indicate that *Enterobacter intermedius* is a senior subjective synonym for the recently described species *Kluyvera cochleae* (A.G. Steigerwalt and J.J. Farmer, unpublished data).

Erwinia Plant isolates of erwiniae have been mainly studied by phytomicrobiologists and phytopathologists. The media, biochemical and other phenotypic tests used for their isolation, enrichment, cultivation, and identification are quite different from those used for other *Enterobacteriaceae*. The 35–37°C incubation temperature used for most other *Enterobacteriaceae* is near, at, or above the maximum growth temperatures of erwiniae. The isolation of erwiniae from humans or animals is rarely reported. It is not known, however, if they are actually rarely occurring, whether their seeming lack of occurrence reflects improper isolation and enrichment procedures, or, if they are isolated, their identification fails. A study using optimal isolation procedures and an optimum incubation temperature would help to resolve this problem. Also needed is a study to characterize all erwiniae by tests and methods used for other *Enterobacteriaceae*. Erwiniae have been shown to be quite diverse on the basis of DNA relatedness studies (Gardner and Kado, 1972; Brenner et al., 1973b; Brenner et al., 1973b, and now 16S rRNA sequence studies (Kwon et al., 1997). Several recently proposed classifications have expanded the genus *Pantoea* to include the Herbicola or Herbicola-Lathyri group of the genus *Erwinia*. Similarly, there have been proposals to change the circumscription of the genus *Pectobacterium* to include a number of species formerly contained in the genus *Erwinia*, and the new genus *Brenneria* was recently described for other species formerly in *Erwinia* (see Table BXII.γ.192 and chapters on *Erwinia*, *Pantoea*, *Pectobacterium*, and *Brenneria*).

Escherichia* and *Shigella The four nomenspecies of *Shigella* and *E. coli* are a single genomospecies on the basis of DNA relatedness (Brenner et al., 1972a, 1973a). *Shigella* and *E. coli* strains are often extremely difficult to separate biochemically since there are aerogenic (gas-producing) shigellae and lactose-negative, anaerogenic, nonmotile *E. coli*. Such strains can cause a dysentery-like diarrhea, so pathogenicity does not provide definitive separation. Shigellae can be considered metabolically inactive biogroups of *E. coli*. Based mainly on molecular data, it has been argued that these organisms do not represent distinct species, but a continuum of closely related lines of descent from a common ancestor. In this *Manual*, they are classified as distinct species because of the ease of communication these names provide in medical microbiology and because of the resistance and confusion that would be caused by reclassification. However, the original usage implying that shigellae were pathogenic and *E. coli* was not is certainly not true. Tamura et al. (1986) proposed an alternative classification. They defined a new genus *Leclercia*, with the single species *L. adecarboxylata*, which was originally named *Escherichia adecarboxylata*.

Hafnia *Hafnia alvei* remains the only named species in this genus. DNA studies revealed two separate DNA relatedness groups within *H. alvei*, with approximately one-half of the strains in each relatedness group (Steigerwalt et al., 1976). A second species was not designated since no single biochemical test or series of tests served to unequivocally separate the two relatedness groups (F.W. Brenner and J.J. Farmer III, personal communication). *"Hafnia protea"* is an illegitimate name for *Obesumbacterium proteus*. There were two biogroups within *O. proteus*. Biogroup 2 is *O. proteus*, whereas biogroup 1 is a metabolically inactive biogroup of *H. alvei* that has become adapted to the brewery environment.

Klebsiella In 1984, Orskov proposed a classification in which *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* would be classified as subspecies of *K. pneumoniae* (Orskov, 1984a). While there is no doubt that these 3 nomenspecies are the same genomospecies on the basis of DNA relatedness studies (Brenner, Steigerwalt, and Fanning, 1972), many laboratories still accept their original classification and report them as separate species. *Klebsiella ornithinolytica* was described by Sakazaki et al. (1989a) for strains previously determined to be a biogroup of *Klebsiella planticola* by Farmer et al., 1985a). These strains have been variously referred to as ornithine-positive *K. oxytoca*, NIH (Japan) Group 12, CDC

TABLE BXII.γ.196. Additional biochemical reactions of *Enterobacteriaceae*[a, b, c]

Species	Nitrate reductase	Tetrathionate reductase	D-Galacturonate	2-keto-Gluconate	γ-Glutamyl transferase
Citrobacter amalonaticus		+		+	+
Citrobacter freundii	+, A	+	+	+	+
Citrobacter koseri		−		+	+
Edwardsiella hoshinae				−	
Edwardsiella tarda	+, B	+		−	−
Erwinia carotovora	+, A	−	+	−	+
Enterobacter aerogenes	+, A	−	+	+	+
Enterobacter cloacae	+, A	−	d	+	+
Enterobacter gergoviae				+	+
Enterobacter sakazakii				+	
Escherichia coli	+, A	−		−	+
Hafnia alvei	d, A or B	d	+	+	+
Klebsiella oxytoca	d, A	d		+	+
Klebsiella pneumoniae subsp. *ozaenae*		−		+	+
Klebsiella pneumoniae subsp. *pneumoniae*	+, A	−		+	+
Klebsiella pneumoniae subsp. *rhinoscleromatis*		−		d	−
Morganella morganii	d, A	+		−	+
Pantoea agglomerans	+, A	−	+	+	+
Proteus mirabilis	+, A	+		−	+
Proteus vulgaris	+, A	+		−	+
Providencia alcalifaciens	+, B	+		−	+
Providencia rettgeri	+, A	+		d	+
Providencia stuartii	+, A	+		−	+
Salmonella enterica subsp. *arizonae*	+, A	+	−	−	−
Salmonella enterica subsp. *diarizonae*	+, A	+	+	−	+
Salmonella enterica subsp. *enterica*	+, A	+	-	−	+
Salmonella enterica subsp. *houtenae*	+, A	+	+	−	+
Salmonella enterica subsp. *salamae*	+, A	+	+	−	+
Serratia ficaria				+	+
Serratia liquefaciens	+, A	+	+	+	+
Serratia marcescens	+, A	d	+	+	+
Serratia odorifera	+, A	−		+	+
Serratia plymuthica	+, A	−	+	+	+
Serratia rubidaea	+, A	−	+	+	+
Shigella boydii	+, A	−		−	d
Shigella dysenteriae		−		−	d
Shigella flexneri	+, A	−		−	d
Shigella sonnei	+, A	−		−	−
Yersinia enterocolitica	+, B	d		d	+
Yersinia pestis	d, B	d		-	−
Yersinia pseudotuberculosis	+, B	−		−	+
Yersinia frederiksenii	+, B	+			
Yersinia intermedia	+, A or B	+			
Yersinia kristensenii	+, B	d			

[a]Symbols: +, 90% or more of strains are positive; d, 10.0–89.9% positive; −, 0–9.9% positive; blank space, not available; A, type A nitrate reductase; B, type B nitrate reductase.

[b]The nitrate reductase test was incubated at 32°C; all other tests were incubated at 35–37°C. The γ-glutamyl transferase test was read at 24 h; all other test results were read at 48 h.

[c]Data compiled from: Pichinoty et al. (1969), Grimont (1977b), Richard (1977), Grimont et al. (1978a), Le Minor et al. (1979), Bercovier et al. (1980a), Bercovier et al. (1980b), Brenner et al. (1980e), Giammanco et al. (1980), Ursing et al. (1980a), and Buissière et al. (1981).

Klebsiella Group 47 (indole-positive, ornithine-positive); and indole-positive, ornithine-positive biogroup of *Klebsiella planticola*. Laboratories agreed on the phenotypic properties of this organism, but they disagreed on whether DNA relatedness data indicated that it should be retained as a biogroup of *K. planticola* or that it should be a separate species.

Kluyvera The recently described species *K. cochleae* (Müller et al., 1996) appears to be a junior subjective synonym for *Enterobacter intermedius* (A.G. Steigerwalt and J.J. Farmer, unpublished data). In this *Manual*, this species is not included as one of the species of *Kluyvera*, but see *Enterobacter intermedius*.

Obesumbacterium proteus. See *Hafnia* above.

Pantoea In this *Manual*, the genus *Pantoea* includes species previously contained in the *Enterobacter agglomerans* complex (including *Erwinia herbicola*, *Erwinia lathyri*, *Erwinia ananas*, *Erwinia uredovora*, *Erwinia milletiae*, and *Erwinia stewartii*). Future proposals may expand its definition to include additional species to be defined from some of the remaining *Enterobacter agglomerans* DNA hybridization groups that have not yet been classified (Brenner et al., 1984a; Grimont and Grimont, 1992). See sections on *Enterobacter* and *Erwinia* above.

Photorhabdus* and *Xenorhabdus Substantial differences in biochemical reactions have been reported by different laboratories (Grimont et al., 1984b; Akhurst and Boemare, 1988; Farmer et al., 1989). Many of the differences are probably due to slow and weak reactions and to differences in media. In the proposed classification of Akhurst and Boemare, *Xenorhabdus beddingii* and *Xenorhabdus bovienii*, are classified as species (Akhurst and Boemare, 1988). They were originally described as subspecies of *Xenorhabdus nematophilus*. Four additional genomospecies within

Photorhabdus luminescens (*Xenorhabdus luminescens*) were not named because they are not phenotypically separable from *P. luminescens* (Farmer et al., 1989).

Proteus The type strain of *Proteus vulgaris* is in a DNA relatedness group that includes only one other strain out of 36 tested. The remaining strains are in four new genomospecies, which include essentially all isolates currently identified as *P. vulgaris* (Brenner et al., 1995). A request for an opinion to replace the type strain was submitted (Brenner et al., 1995), and was recently granted (H. Trüper, personal communication). MacDonell and Colwell (1985) recommended that *Plesiomonas shigelloides* be classified in the genus *Proteus* because its 5S rRNA sequence is closely related to that of *P. mirabilis*. They did not formally describe the new combination (*"Proteus shigelloides"*). Such a change is not supported by phenotypic characteristics or by DNA relatedness and the new combination has not been used.

Salmonella The classification and nomenclature of salmonellae has long been a source of confusion, even to specialists. Much of the confusion was due to the practice of naming serotypes (serovars) and equating them with species. All phylogenetic data indicate that the evolution of the genus *Salmonella* is a continuum, but several recent classifications recognize two distinct species of *Salmonella*, one of which contains six subspecies (Reeves et al., 1989a; Brenner and McWhorter-Murlin, 1998). The type species of the genus is *Salmonella choleraesuis*. Unfortunately, there is also a serotype Choleraesuis that, until recently was treated as a species. For this reason Le Minor and Popoff (1987) requested an opinion to replace the name *S. choleraesuis* with *"Salmonella enterica"*. This request was not approved by the Judicial Commission (Wayne, 1994; Judicial Commission, 1991), although the wording of the refusal seemed to leave the door open for another request to remedy the confusion caused by the name *S. choleraesuis*. This request has now been resubmitted by Euzéby (1999), who also requested that *"Salmonella typhi"* be designated as a species. In the interim, the WHO International Collaborating Centre for *Salmonella* located at the Pasteur Institute in Paris, the National *Salmonella* Center located at the Centers for Disease Control and Prevention in Atlanta, and many other reference laboratories and their constituents have begun to use *S. enterica*, so both names are currently in use. In one proposed classification there are 6 subspecies of *S. choleraesuis*: subsp. *enterica* (or subspecies I or subspecies 1), for those biochemically typical serotypes previously placed in subgenus I ; subsp. *salamae* (or subspecies II or subspecies 2) , for biochemically atypical serotypes previously in subgenus II; subsp. *arizonae* (or subspecies IIIa or subspecies 3a) for the monophasic strains previously in *"Arizona hinshawii"* or subgenus III; subspecies subsp. *diarizonae* (or subspecies IIIb or subspecies 3b) for the diphasic strains previously in *"A. hinshawii"* or subgenus III; subsp. *houtenae* (or subspecies IV or subspecies 4) for the biochemically atypical strains previously in subgenus IV; and subsp. *indica* (or subspecies VI or subspecies 6) for more recently defined atypical strains that differ from the biochemical patterns of subspecies I-IV. The second species is *Salmonella bongori*, a small number of biochemically unique serotypes that have been classified as subsp. *bongori* (or subspecies V or subspecies 5) but are considered to be separate species in the proposed classification of Reeves et al., (1989a) and in this *Manual*. The practice in medical bacteriology is to use names for the serotypes in subsp. *enterica*. For example, the serotypes formerly reported as *S. typhi* and *S. typhimurium* would now be reported as *S. choleraesuis* (or *"S. enterica"*) subsp. *enterica* (or I or 1) serotype Typhi or Typhimurium. Note that the serotype name is capitalized and nonitalicized. Alternatively these serotypes could be reported simply as *Salmonella* serotype Typhi and *Salmonella* serotype Typhimurium. Serotypes in the other subspecies are reported similarly. Some laboratories choose to use names (where the serotypes are named) and some choose to use the antigenic formulae. Serotypes in subsp. *arizonae* and subsp. *diarizonae* are not named. Therefore a typical report, would be *Salmonella* serotype 60:k:z (= *Salmonella enterica* subsp. *diarizonae* serotype 60:k:z). *Salmonella enterica* subsp. *indica* serotype Brookfield has the antigenic formula 66:z41:-. Any of the following reports would be accurate: *Salmonella* serotype Brookfield, *Salmonella* subsp. *indica* serotype Brookfield, *Salmonella* subsp. V Brookfield, as would any of the above designations with the antigenic formula used instead of the name Brookfield. Much of the literature still uses the "serotype as species" classification and uses the Linnean system of writing the genus and species in italics. Examples include *Salmonella typhi*, *Salmonella typhimurium*, and *Salmonella enteritidis*.

Yokenella *Yokenella*, with its single species *Y. regensburgei*, was described by Kosako et al. in 1984. The genus *Koserella* with its single species *K. trabulsii*, was described by Hickman-Brenner et al. in 1985a *Y. regensburgei* and *K. trabulsii* were shown to be subjective synonyms by Kosako et al. in 1987 Other synonyms for this organism are *Hafnia* hybridization group 3, Enteric Group 45, NIH (Japan) biogroup 9. Even though *K. trabulsii* had priority by virtue of its earlier alphabetical appearance on Validation List No. 17 in the *International Journal of Systematic Bacteriology*, both groups of investigators agreed that *Yokenella* should be the genus name.

Unnamed Enteric Groups A number of groups belonging to the family *Enterobacteriaceae* have been characterized in the Enteric Reference Laboratory at the CDC but not given genus and species names. Four Enteric Groups are briefly described below. Their biochemical profiles are included in Table BXII.γ.193.

Enteric Group 58 Enteric Group 58 consists of strains that were first recognized in 1981 and described in 1985 (Farmer et al., 1985a). The first five isolates were from human clinical specimens; four from wounds (foot, ankle, leg, and hip), and one from feces. Since the original report in 1985, the number of isolates at CDC has grown to 21, including an isolate from a case of bacteremia. Enteric Group 58 has the general properties of the family *Enterobacteriaceae*.

Enteric Group 60 Enteric Group 60 consists of four strains first recognized in 1981 and described in 1985 (Farmer et al., 1985a). All of the strains were from human clinical specimens (three from urine and one from sputum). Enteric Group 60 is inactive biochemically and was originally thought to be most like *Morganella morganii*. However, it is sensitive to colistin and tyrosine-negative, reactions incompatible with *Morganella*. Enteric Group 60 has the general properties of the family *Enterobacteriaceae*. Its closest phylogenetic relatives are not yet known.

Enteric Group 68 Enteric Group 68 consists of a small group of strains isolated from human urine that were first recognized in 1981 and described in 1985 (Farmer et al., 1985a). The group is positive for DNase, but otherwise quite different from *Serratia*. Enteric Group 68 also has the general properties of the family *Enterobacteriaceae*. Its closest phylogenetic relatives are not yet known.

Enteric Group 69 Enteric Group 69 is the name given to a group of strains isolated from refrigerated beef carcasses (Farmer et al., 1985a). The strains were phenotypically similar to *E. sa-*

kazakii in most biochemical reactions, including production of a yellow pigment. However, they were only 43% related to *E. sakazakii* by DNA–DNA hybridization. Recent DNA relatedness data by Kosako et al. (1996) indicated that *Enterobacter kobei* is the closest relative of Enteric Group 69.

Genus I. **Escherichia** *Castellani and Chalmers 1919, 941T[AL]*

FLEMMING SCHEUTZ AND NANCY A. STROCKBINE

Esch.er.i'chi.a. M.L. fem. n. *Escherichia* named after Theodor Escherich, who isolated the type species of the genus.

Straight cylindrical rods, 1.1–1.5 × 2.0–6.0 μm, occurring singly or in pairs. Conform to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile by peritrichous flagella or nonmotile. Aerobic and facultatively anaerobic having both a respiratory and a fermentative type of metabolism, but anaerogenic biotypes occur. Oxidase negative. Chemoorganotrophic. **Both acid and gas are formed from most fermentable carbohydrates, but *i*-inositol is not utilized and D-adonitol is utilized only by *Escherichia fergusonii*. Lactose is fermented by most strains of *Escherichia coli*,** but fermentation may be delayed or absent in *Escherichia blattae*, *Escherichia hermannii*, *Escherichia fergusonii*, and *Escherichia vulneris*. **Do not grow in KCN (with the exception of *E. hermannii* and a small proportion of *E. vulneris*). Usually do not produce H_2S.** *E. coli* occur naturally in the lower part of the intestine of warm-blooded animals, *E. blattae* in the hind-gut of cockroaches, and *E. fergusonii*, *E. hermannii*, and *E. vulneris* are found in the intestine, as well as extraintestinal sites of warm-blooded animals. Seven copies of the *rrn* operon with genes coding for 16S, 23S, and 5S rRNA are present on the chromosome of *E. coli*. Comparative sequence analysis between the genes for 16S rRNA of *E. coli*, *E. vulneris*, and *E. hermannii* and homologous genes from all eubacteria places *E. coli* and *E. vulneris* together in a tightly related cluster with shigellae, and *E. hermannii* between *Salmonella* spp. and *Citrobacter freundii* (Cilia et al., 1996). Based on 16S rRNA sequencing, escherichiae belong in the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 48–59.

Type species: **Escherichia coli** (Migula 1895) Castellani and Chalmers 1919, 941 (*Bacillus coli* Migula 1895, 27.)

FURTHER DESCRIPTIVE INFORMATION

Cell morphology Escherichiae are straight, cylindrical, Gram-negative rods with rounded ends that are 1.1–1.5 μm in diameter and 2.0–6.0 μm in length. They occur singly or in pairs and can be motile by peritrichous flagella or nonmotile. Fig. BXII.γ.191 shows negatively stained preparations of each of the *Escherichia* species. See Nanninga (1985) for a comprehensive treatment of the ultrastructure of *E. coli*.

Phylogenetic and systematic treatment The genus consists of five species: *E. coli*, *E. hermannii*, *E. fergusonii*, *E. vulneris*, and *E. blattae*. Biochemical reactions that will help in differentiating between the species of *Escherichia* are listed in Table BXII.γ.197.

Biochemical reactions Escherichiae produce strong acids and usually gas from the fermentation of D-glucose (positive in the Methyl Red test) and do not produce acetyl-methyl carbinol (acetoin) (negative in the Voges–Proskauer test). Sodium acetate is frequently used as a sole carbon source, except by *E. blattae* and a majority of *E. vulneris* strains. Citrate (Simmons' citrate agar) cannot be used by *E. coli* and *E. vulneris*, whereas a smaller proportion of *E. fergusonii* and *E. hermannii* exhibit immediate or delayed use of this substrate. Growth on Simmons' citrate agar by *E. blattae* is variable and probably strain specific.

Lysine is decarboxylated by the majority of strains. Exceptions include "metabolically inactive" *E. coli* strains, the majority of enteroinvasive *E. coli* strains (EIEC), and *E. hermannii*. Ornithine is decarboxylated by all species except by *E. vulneris* and a little less than half of *E. coli* strains. Indole is produced by all species except *E. blattae* and *E. vulneris*.

Other tests that help in differentiating between the species of *Escherichia* include growth in potassium cyanide, malonate utilization, and acid production from D-adonitol, D-arabitol, cellobiose, dulcitol, lactose, D-mannitol, melibiose, D-sorbitol, and mucate. See Table BXII.γ.197.

Molecular data Findings from the comparison of 16S rDNA sequences performed with strains of *E. coli*, *Salmonella* spp., and *C. freundii* have shown a close phylogenetic relatedness between these bacteria (Ahmad et al., 1990; Cilia et al., 1996; Chang et al., 1997). Analysis of 16S rDNA sequences separates the two *Salmonella* species (*S. enterica* and *S. bongori*) from the complex of *E. coli* and *Shigella*, and shows that *E. hermannii* is more closely related to *Salmonella enterica* and *C. freundii* (Christensen et al., 1998). This analysis is unable to separate inter-operon variation of *E. coli* from strains of *E. coli* and from *Shigella*. Patterns of sequence heterogeneity in strains from the *E. coli* Collection of Reference (ECOR) (Ochman and Selander, 1984) have been located at regions V1 and V6 of cloned 16S rRNA genes (Martinez-Murcia et al., 1999).

Average DNA relatedness assessed by DNA–DNA hybridization among *Escherichia* species ranges from 29% to 94% (Table BXII.γ.198). DNAs from different strains of *E. coli* are closely related (average, 84%; Table BXII.γ.198). With the exception of *S. boydii* serotype 13, the DNAs of *E. coli* and the four *Shigella* species show such a high degree of relatedness (average 80–87%, except *S. boydii* type 13, which is about 65%) that these species should be considered as a single species (Brenner et al., 1973a). The distinction between these bacteria prevails, however, for reasons of historical/medical precedent and to avoid confusion in the literature and with existing surveillance systems.

Based on complete sequencing of the K-12 strain MG1655 (Blattner et al., 1997), it is estimated that the *E. coli* lineage diverged from the *Salmonella* lineage some 100 million years ago (Lawrence and Ochman, 1998). This is a little less than the 120–160 million years estimated by calibration of the rate of 16S rRNA evolution in bacteria (Ochman and Wilson, 1987). The chromosome of *E. coli* strain MG1655 is 4,639,221 bp and contains 4,288 open reading frames (ORFs). Approximately 18% of these ORFs represent genes that have been acquired and have persisted

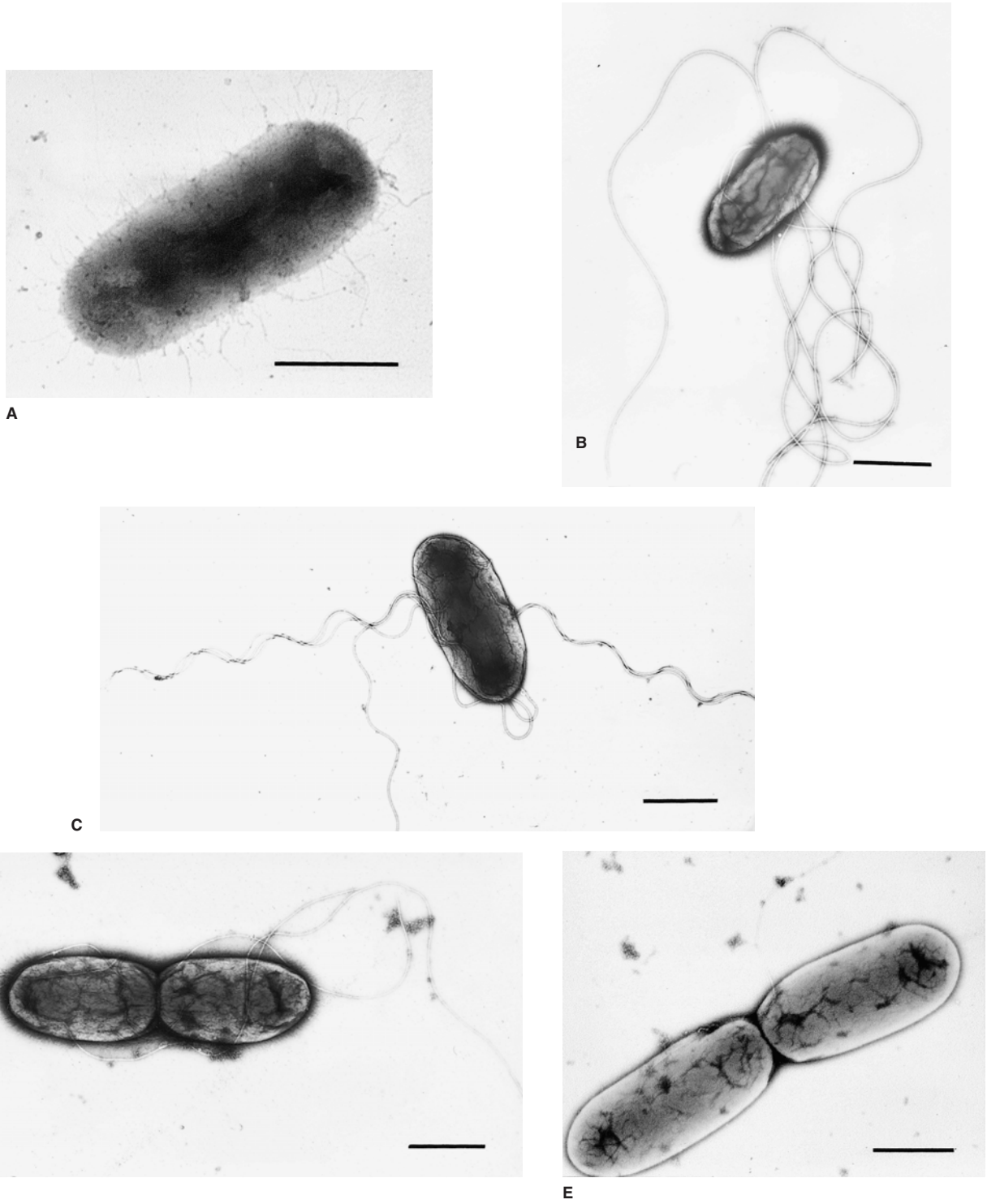


FIGURE BXII.γ.191. Electron micrographs of *E. blattae* strain 2928-78 (*A*), *E. coli* O157:H7 strain EDL933 (*B*), *E. fergusonii* strain 2460-89 (*C*), *E. hermannii* strain 2456-88 (*D*), and *E. vulneris* strain 2485-88 (*E*) prepared by negatively staining in 0.5% (w/v) uranyl acetate. Bar = 1000 nm. (Micrograph courtesy of Charles D. Humphrey, CDC.)

since divergence. This is similar to estimates based on analysis of codon usage, indicating that 16% of sequenced genes arose through horizontal transfer (Médigue et al., 1991). The ability of individual strains and lineages to acquire foreign DNA results in great heterogeneity in both individual genes and in the size of the *E. coli* chromosome. This is exemplified by the unusual serotype O157:H7, the genome of which is 5.5 Mb in size, 859 Kb larger than that of the laboratory strain K-12 (Hayashi et al., 2001; Perna et al., 2001). The genome sizes of 14 *E. coli* strains from the five major subgroups in the ECOR (Ochman and Selander, 1984) range from 4.66–5.30 Mb (Bergthorsson and Ochman, 1995), and the uropathogenic *E. coli* strain J96 genome is

TABLE BXII.γ.197. Differentiation of the five species of *Escherichia*[a,b]

Test	*E. coli*	*E. coli* (metabolically inactive strains)	*E. blattae*	*E. fergusonii*	*E. hermannii*	*E. vulneris*
Indole	+	[+]	−	+	+	−
Citrate, Simmons	−	−	d	[−]	−[c]	−
Lysine decarboxylase	+	d	+	+	−[d]	[+]
Ornithine decarboxylase	d	[−]	+	+	+	−
Motility	+	−	−[e]	+	+	+
KCN, growth	−	−	−	−	+	[−]
Malonate utilization	−	−	+	d	−	[+]
D-Glucose, gas	+	−	+	+	+	+
Acid production from:						
D-Adonitol	−	−	−	+	−	−
D-Arabitol	−	−	−	+	−	−
Cellobiose	−	−	−	+	+	+
Dulcitol	d	d	−	d	[−]	−
Lactose	+	[−]	−	−[f]	d	[−][f]
D-Mannitol	+	+	−	+	+	+
Melibiose	[+]	d	−	−	−	+
D-Sorbitol	+	d	−	−	−	−
Mucate	+	d	d	−	+	[+]
Acetate utilization	+	d	−	+	[+]	d
Yellow pigmentation	−	−	−	−	+	d

[a]Data compiled from references Farmer (1999), Cowan et al. (1995), Holt et al. (1994), and Richard (1989). Reactions for indole for *E. fergusonii* and melibiose for *E. coli* differ slightly in these references. The reactions listed in this table are supported by our own unpublished data.

[b]Symbols: −, 0–10% positive; [−], 11–25% positive; d, 26–75% positive; [+], 76–89% positive; +, 90–100% positive. Results are for 48 h incubation at 36° ± 1°C.

[c]Delayed positive in approximately a fifth of *E. hermannii* strains.

[d]Delayed positive in a third of *E. hermannii* strains.

[e]75% of *E. blattae* strains will become motile after incubation of more than 2 d.

[f]Delayed positive in approximately two thirds of *E. fergusonii* and *E. vulneris* strains.

TABLE BXII.γ.198. DNA relatedness among escherichiae[a]

Labeled DNA from	Average percent relatedness				
	E. coli	*E. blattae*	*E. fergusonii*	*E. hermannii*	*E. vulneris*
E. coli	84	42	64	38	43
E. blattae		90		39	29
E. fergusonii	57		94	59	
E. hermannii	43	32		89	33
E. vulneris	39	29	33	36	78

[a]Data compiled from Ewing, 1986b.

reported to be 5.12 Mb (Melkerson-Watson et al., 2000) as estimated by pulsed field gel electrophoresis (PFGE).

Of the five species in the genus *Escherichia*, *E. coli* is the most studied. Because the amount of knowledge about the other four species is limited, the descriptive information to follow applies to *E. coli*, unless indicated otherwise.

Cell wall composition The chemical composition and molecular structure of the cell wall of *E. coli* has been extensively studied and is described in detail by Neidhardt and Umbarger (1996) and Park (1996). The structural rigidity of the cell wall is provided by the murein sacculus, which consists mainly of a single monomolecular sheet of murein, a complex polymer composed of roughly equal amounts of polysaccharides (*N*-acetylglucosamine [GlcNAc] and *N*-acetylmuramic acid [MurNAc]) and peptides (L-alanine, D-glutamic acid, L-*meso*-diaminopimelic acid (DAP), and D-alanine). Linear chains of alternating units of GlcNAc and MurNAc are linked together by β-1→4 glycosidic bonds, and short chains of the above peptides in alternating D and L optical isomers are attached to the sugars through amide linkages to the carboxyl groups of each muramic acid. Adjacent glycan strands are cross-linked to each other through the peptide side chains to create one giant molecule that provides structural support to the cell. Notable features of the murein of *E. coli* include the presence of a small percentage of peptide chains that either lack the D-alanine or terminate in an additional D-alanine and the absence of amidation involving the carboxyl groups of glutamic acid and DAP. A molecule of lipoprotein is also attached to about every tenth muropeptide.

Outer membrane The outer membrane of an average *E. coli* cell contains over a million molecules of lipopolysaccharide (LPS), which consist of three covalently linked domains: (1) lipid A (endotoxin), (2) the core region of phosphorylated nonrepeating oligosaccharides, and (3) the O antigen polymer of immunogenic repeating oligosaccharides (1–40 units).

Fine structure

Flagella Motile organisms of the genus typically possess 5–10 flagella per cell, which are randomly situated around the cell surface (peritrichous flagellation). The flagellar filament is about 20 nm in diameter and may be up to 20 μm long. It consists of subunits of a single protein, flagellin, which is encoded by the *fliC* gene. Fifty-three antigenically distinct types of flagellin have been described (Ørskov and Ørskov, 1984a). Electron microscopic studies of these antigenically distinct types of flagellar

filaments revealed differences in surface structure; six unique flagellar morphotypes have been described (Lawn et al., 1977). Unlike *Salmonella*, most *E. coli* strains have only one flagellin gene and do not undergo phase variation. Exceptions have been described (Ratiner, 1967, 1982, 1999). See Macnab (1996) for a complete description of the structure and genes involved in motility.

Fimbriae In addition to the proteinaceous flagella, most strains have fimbriae (pili) or fibrillar proteins often extending in great numbers from the bacterial surface and far out into the surrounding medium. A typical *E. coli* K-12 cell contains 100–500 type 1 fimbriae arranged peritrichously, each with a diameter of approximately 7 nm and a length of 0.2–2.0 μm. More than 30 different fimbriae have been described in *E. coli*, which commonly expresses more than one type at a time. The characteristics, biogenesis, and classification schemes of fimbriae are reviewed by Low et al. (1996). Fimbriae have historically been classified by phenotypic properties. One widely used scheme classifies fimbriae according to their adhesive properties for red blood cells from different host species in the presence of mannosides. By this method, two main types of fimbriae are recognized: mannose-sensitive (MS) fimbriae, which are unable to agglutinate red blood cells in the presence of α-D-mannose, and mannose-resistant (MR) fimbriae, which are able to agglutinate red blood cells in the presence of this sugar. MS fimbriae, which include the so-called type 1 fimbriae (pili), are found in the majority of *E. coli* strains and comprise a group of more or less serologically related antigens. Because they are expressed by pathogens as well as commensal organisms, their role in virulence has been difficult to establish. Evidence for the role of type 1 fimbriae in virulence is reviewed by Abraham and Jaiswal (1997). Type 1 fimbriae mediate avid bacterial attachment to mucosal surfaces, to noncellular host constituents, and to various inflammatory cells. They bind to certain oligomannoside-containing glycoproteins present on mucosal surfaces, including the Tamm–Horsfall glycoprotein, which is synthesized in the kidney and present in urinary slime (Ørskov et al., 1980a); fibronectin, a glycoprotein that is a member of a family of proteins found in the extracellular matrix (ECM), plasma, and other body fluids; and laminin, a glycoprotein present in basement membranes (Kukkonen et al., 1993). The genes involved in the synthesis and regulation of type 1 fimbriae are located on the chromosome. Expression of type 1 fimbriae is subject to being turned on or off (phase variation) as a result of the inversion of a 314 base-pair fragment of DNA containing the promoter region of the gene encoding the major fimbrial subunit (*fimA*). Expression of type 1 fimbriae is influenced by environmental and growth conditions and is controlled by global regulatory factors such as leucine-responsive regulatory protein (Lrp), integration host factor (IHF) and histone-like protein H-NS. See Abraham and Jaiswal (1997) and Low et al. (1996) for a review of the environmental factors and genes involved in synthesis and regulation of type 1 fimbriae.

MR fimbriae are serologically diverse (Ørskov et al., 1980b, 1982; Ørskov and Ørskov, 1990) and often function as virulence factors to mediate adherence that is species- and organ-specific. The genes for these proteins may be located on plasmids or on the chromosome. When located chromosomally, they often cluster together with other virulence genes in regions of the chromosome referred to as pathogenicity islands (PAIs). More than any other virulence factor, the MR pili of *E. coli* illustrate the species' capacity to adapt to the receptor-specific epithelial cells of certain hosts primarily through horizontal acquisition of gene cassettes on plasmids, phages, or other mobile DNA elements that will allow for colonization. Evidence for the horizontal transfer of fimbrial genes in *E. coli* comes from the remarkable similarity between the genetic organization of its fimbrial operons and those of other members of the family *Enterobacteriaceae* and from the strikingly low C + G content and different codon usage pattern among the fimbrial genes compared to those observed overall among other genes on the *E. coli* chromosome.

Fimbriae may also be classified based on their morphology. One group consists of thick, rod-shaped fimbriae with a diameter of 7 nm (range 3.4–8 nm), a length of 0.5–2 μm, and an axial hole diameter of 2.0–2.5 nm. Fimbriae with these dimensions are represented by the rigid pyelonephritis (P), sialic acid (S), type 1, F6 (987P), colonization factor antigen I (CFA/I), coli surface antigen 1 (CS1) and CS2 pili, and by the bundle-forming CS8 (CFA/III) and CS21 (longus), the latter with homology to the type 4 fimbrial family. Enteropathogenic *E. coli* (EPEC) is known to produce a type 4 fimbria called the bundle-forming pilus (BFP). Another flexible, bundle-forming fimbrial structure of 2–3 nm diameter, designated aggregative adherence fimbriae I (AAF/I), shows no homology to the type 4 class of fimbriae. Together with another recently described AAF/II fimbria, it exhibits sequence and organizational resemblance to the Dr family. Another group consisting of thinner, more flexible fibrillae with a width of 2–5 nm and a length of 0.5–2.0 μm is represented by F4 (K88), F5 (K99), F41, and CS3. Helical fibrillae, where two fibrillae are arranged in a helix, are represented by CS5 and CS7.

Nonfimbrial and related adhesins Bacterial adherence may also be mediated by adhesions that are afimbrial (AFA) or nonfimbrial (NFA). Some of these proteins form larger multimers that aggregate around the bacterial cell as an amorphous structure reminiscent of capsular K antigens. Afimbrial adhesins are found in both uropathogenic *E. coli* (UPEC) and in diffusely adhering *E. coli* (DAEC) and represented by the Afa/Dr family consisting of Dr (previously referred to as O75X) and Dr-II (*drb*), the F1845 pilus (*daa*), and AFAI-IV (*afa*). Dr fimbriae and related adhesins recognize different epitopes of the Dr blood group antigen (Nowicki et al., 1990) and bind to a complement-regulatory protein, the common receptor decay accelerating factor (DAF). *E. coli* strains expressing Dr fimbriae are able to enter epithelial cells by interacting with DAF (Goluszko et al., 1997). Other adhesins such as the M agglutinin and AIDA-I adhesin, a plasmid-encoded outer membrane protein involved in diffuse adherence of certain types of *E. coli* (Benz and Schmidt, 1989), are commonly present.

Colonial and cultural characteristics; life cycles Depending on the degree of polymerization of the O antigen polysaccharide, the phenotypes of strains growing on agar media are described as smooth (S) or rough (R). S forms, which usually grow on nutrient agar as convex, glistening, moist, gray colonies (2–3 mm diameter) with a defined edge or in fluid medium as turbid growth, have developed polysaccharide side chains, while R forms, which usually grow as flat, dry, dull, wrinkled colonies (1–5 mm diameter) with a blurred edge on agar and agglutinate spontaneously in fluid media, have lost their polysaccharide side chains by mutation (Lüderitz et al., 1966). There are intermediate forms between these extremes. Mucoid and slime-producing forms occur. *E. hermannii* is yellow-pigmented, as are half of the described *E. vulneris* strains. See Raetz (1996) and Hull

(1997) for a discussion of the chemical structure, biosynthesis, and biological/virulence properties of LPS.

Nutrition and growth conditions Of the range of temperatures, pH values, water activities, and pressures over which bacterial growth can occur, *E. coli* strains survive and grow over the mid-range (15–45°C) of these environmental conditions. Most strains can grow over a temperature range of approximately 40°C. The normal temperature range for balanced growth extends from 21° to 37°C; however, strains that can grow at temperatures as low as 7.5–7.8°C (Shaw et al., 1971) and as high as 49°C (Herendeen et al., 1979) have been described. A minimum growth temperature for *E. vulneris* of 1.6°C (0.8–2.6°C) was reported for a strain isolated from refrigerated meat (Ridell and Korkeala, 1997). *E. coli* is neutrophilic and will grow over the mid-range of pH, from about pH 5.0 to 9.0 (Ingraham and Marr, 1996).

Metabolism and metabolic pathways Glucose and other carbohydrates are fermented with the production of pyruvate, which is further converted into lactic, acetic, and formic acids. Part of the formic acid is split by a complex hydrogenlyase system into equal amounts of CO_2 and H_2.

Phylogeny The establishment of the *Escherichia coli* Collection of Reference (ECOR) in 1984 (Ochman and Selander, 1984) and subsequent studies of the strains in ECOR and comparisons with other *E. coli* strains have contributed substantially to our understanding of the evolution and population structure of *E. coli*. ECOR is a collection of 72 strains from humans and 16 other mammalian species from various geographical areas that have been grouped into five main groups—A, B (comprising subgroups B1 and B2), C, D, and E—according to their electrophoretic types and enzyme allele (allozyme) profiles, based on the results of multilocus enzyme electrophoresis (MLEE) (Selander et al., 1986). The original data for 35 enzymes (Selander et al., 1987) have been expanded to include allozymes of four esterase loci (Goullet and Picard, 1989), and, based on allelic variation at 38 enzyme-encoding loci, multilocus genotypes have been used to construct a dendrogram based on the neighbor joining algorithm (Herzer et al., 1990), demonstrating the clonal structure of the species. The phylogenetic groups are distinguishable but not identical by random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) of *rrn* genes (ribotyping) (Desjardins et al., 1995), and to a lesser extent by repetitive-element PCR (rep-PCR) fingerprinting using ERIC2 and BOXA1R primers (Johnson and O'Bryan, 2000). Generating a phylogenetic tree of the ECOR and 15 O157 strains by fluorescent amplified-fragment length polymorphism (FAFLP) demonstrated close correlation with the MLEE groups of ECOR and placed the STEC/VTEC O157 strains on an outlier branch (Arnold et al., 1999). Thus, there is sufficient evidence that the ECOR strains broadly represent genotypic variation in clonal groups of *E. coli* in spite of the fact that many isolates are commensal forms from healthy carriers and that only 11 out of the 72 strains are from human disease: 1 strain from a case of asymptomatic bacteriuria, 4 from acute cystitis, and 6 from acute pyelonephritis. Other MLEE studies have showed clonal relationships of diarrheagenic *E. coli*, and a collection of 78 diarrheagenic *E. coli* (DEC) representing 15 clonal groups has been established (Whittam et al., 1993). A similar collection of STEC/VTEC strains has been collected by the STEC Center based at the National Food Safety and Toxicology Center at Michigan State University and is designed to facilitate research on the Shiga/Verocytotoxin producing *E. coli* by providing a standard reference collection of well-characterized strains and a central, on-line accessible database.*

Numerous studies have indicated that *E. coli* and the four named species of *Shigella* should be regarded as being one species (Brenner et al., 1972a, b, 1973a; Goullet, 1980; Ochman et al., 1983; Whittam et al., 1983; Hartl and Dykhuizen, 1984; Karaolis et al., 1994; Stevenson et al., 1994; Whittam, 1996; Pupo et al., 1997). Findings from MLEE studies combined with those from *mdh* (malate dehydrogenase) housekeeping gene sequence studies (Pupo et al., 1997) and ribotyping (Rolland et al., 1998) confirm that the genus *Shigella* comprises a group of closely related pathogenic *E. coli* strains and indicate that *Shigella*, EIEC, and other diarrheagenic *E. coli* strains do not have a single evolutionary origin, but are derived from different ancestral strains many times. Furthermore, pathogenic strains belonging to pathogenic groups of EIEC, EPEC, and ETEC were found to be closely related to ECOR group A strains by MLEE and have *mdh* sequences identical to five ECOR strains from group A, which is thought to represent commensal strains. It has been suggested that any *E. coli* strain may acquire virulence factors from numerous sources, including plasmids, bacteriophages, and other mobile DNA elements from a large pool of strain-specific genes whose origin could be outside the species boundaries, and that this is how a commensal form is turned into a pathogenic form (Pupo et al., 1997; Hurtado and Rodriguez-Valera, 1999; Donnenberg and Whittam, 2001). In their analysis, Lawrence and Ochman (1998) surmised that about 10% of the *E. coli* K-12 genome consists of genes that were acquired in over 200 events of lateral gene transfer, which occurred subsequent to the divergence of *E. coli* and *Salmonella* some 100 million years ago. While mutations have contributed substantially to the heterogeneity of *E. coli*, the importance of these recombinational events should not be underestimated, and transfer of smaller or bigger segments of DNA between different clonal lineages and other species has probably contributed more to the evolution of *E. coli* than anyone could have imagined. Ongoing and future studies will be directed toward increasing our understanding of the principles that govern gene patterns and the relations between individual traits in terms of their significance for virulence and host adaptation. The combined efforts should contribute to a general IDEA (Index of Diversity in Evolution and Adaptation) that will bridge the phylogenetic approach based on the clonal concept on one side and the horizontal transfer of gene cassettes on the other side.

Mutants, plasmids, phages, and bacteriocins The fact that *E. coli* mutants can easily be produced in the laboratory has substantially contributed to our understanding of many genetic mechanisms, ranging from the characterization and function of an individual gene to the description of complex operons. Plasmids carrying resistance genes (R plasmids) have been used as vectors and introduced into both laboratory strains and wild-type strains, and phages and other mobile elements such as transposons have been used widely in both research and applied biotechnology. Virulence genes are often found on plasmids (see pathogenicity section below). *E. coli* strains produce a variety of secreted antibiotically active polypeptides, bacteriocins, and microcins, which have the ability to kill or inhibit competing bacterial strains. Colicins, encoded by plasmids of *E. coli*, act on other

**Editorial Note:* At the time of publication, this information could be obtained at http://www.shigatox.net.

E. coli or closely related bacteria that do not carry that particular Col plasmid. Small lipoproteins are important components of the secretory apparatus, which facilitates release of colicin and plasmid- and phage-encoded proteins across the outer membrane. For additional information on *E. coli* mutants, plasmids, and phages see Campbell (1996), Bachmann (1996), Helinski et al. (1996), and Harwood (1993).

Antigenic structure

O antigens The main aspect of this analysis is the O antigen determination based on antigenicity of the LPS; O group designations run from O1 to O173 but O groups O31, O47, O67, O72, O93, O94, and O122 have been removed. Also included are the provisional O groups OX3 and OX7 listed by Ewing (1986b), which will receive the designations O174 and O175, respectively. An additional six new O groups representing STEC/VTEC strains are currently being investigated and will receive O designations O176 through O181 (Scheutz, unpublished data). Subtypes exist within most O groups and are designated ab, ac, etc., e.g., O128ab and O128ac. Many of these O antigens cross-react with other O antigens and to some extent to K antigens within *E. coli*, with other members of the genus and with other enterobacteria. Recent molecular typing using primers just outside the O antigen gene cluster (*rfb*) of 148 representative O groups observed unique amplified fragments for each O group with sizes ranging from 1.7 to 20 kb (Coimbra et al., 2000). Subsequent *Mbo*II digestion of PCR-amplified products resulted in clearly identifiable and reproducible O patterns for the great majority of O groups with a variation of band numbers for each pattern ranging from 5 to 25. Computer analysis identified a total of 147 O patterns and allowed subdivision of 13 O groups. However, two or more O groups shared a pattern among 13 other O patterns. The restriction method (*rfb*-RFLP) is more rapid and may prove to be more sensitive than conventional serotyping since 100% of strains are typeable, particularly those that are O rough or nonagglutinating. Additionally, it should facilitate the typing of strains outside the existing O antigen scheme, which is restricted to include only O groups of clinical, epidemiological, or scientific relevance. The success and general application of such a typing scheme will require international collaboration to develop standardized methods for generating, comparing, and maintaining a database of O patterns.

K antigens The K antigens are the acidic capsular polysaccharide (CPS) antigens. K antigens may be separated into two distinct groups designated group I and group II. Group I antigens, which are composed of high-molecular mass (>100 kDa) CPS, are only found in strains with O groups O8, O9, O20, and O101, and are expressed at both 18° and 37°C. Group I antigens are subdivided according to the absence (IA) or presence (IB) of amino sugars on their CPS. The CPSs of group IA antigens share structural identity or resemblance to those from *Klebsiella* spp., whereas the CPSs of group IB antigens share no structural resemblance to those from other bacteria. Representative strains expressing group IA antigens do not contain the *rol* (*cld*) gene encoding the regulator of lipopolysaccharide O-chain length, whereas a similar subset of strains expressing group IB antigens contains the *rol* gene (Dodgson et al., 1996). Portions of the CPSs of some group I antigens are attached to the lipid A-core in a form that has been designated K_{LPS}, which will behave similarly to the traditional O antigens. A good example of a group I K_{LPS} is K84, which may be operationally defined as an O antigen and was originally designated as O93.

Group II antigens, which are composed of low molecular mass (<50 kDa) CPS, are found primarily in strains with O groups that are associated with extraintestinal disease. The CPSs of many group II antigens have structural resemblance or near identity to those from Gram-positive bacteria. These antigens differ widely in composition and structural features and may be divided into subgroups based on their acidic components. Twenty to fifty percent of the CPS chains are bound to phospholipids. They were originally thought to be temperature dependent, i. e., only expressed at 37°C. However, K2, K3, K10, K11, K19, K54/K96, and K98, which are tentatively classified as group I/II antigens (Finke et al., 1990), show no temperature regulation of their capsules and, like group I antigens, do not depend on an elevated CMP-KDO concentration for capsule expression. Based on genetic data, a subset of the group I/II antigens (K3, K10, and K54/K96) has been designated group III antigens (Pearce and Roberts, 1995). A number of K antigens are closely related (indicated below by "~") or identical (indicated below by "="). The CPSs of some group IB antigens are structurally identical to the side chains of O antigens and are only considered as K antigens when co-expressed with another authentic O antigen. The following 60 different K antigens are recognized: K1, K2a/ac, K3, K4, K5, K6, K7 (=K56), K8, K9 (=O104), K10, K11, K12 (=K82), K13 (~ K20 and ~ K23), K14, K15, K16, K18a, K18ab (=K22), K19, K24, K26, K27, K28, K29, K30, K31, K34, K37, K39, K40, K41, K42, K43, K44, K45, K46, K47, K49 (=O46), K50, K51, K52, K53, K54 (~ K96), K55, K74, K84, K85ab/ac (=O141), K87 (=O32), K92, K93, K95, K97, K98, K100, K101, K102, K103, KX104, KX105, and KX106. The inclusion of an "X" before the number represents a temporary K antigen designation. A description of the serology, chemistry, and genetics of *E. coli* O and K antigens is given by Ørskov et al. (1977).

H antigens Flagellar or H antigens make up the third main group of serotyping antigens. A total of 56 H antigens have been described, but two, H13 and H22, have been removed as being *C. freundii*, and H50 has been withdrawn because it is identical to H10. Cross-reactions are also seen between the H antigens.

Fields et al. (1997) described a tentative molecular method for the differentiation of flagellar antigen groups in *E. coli* based on restriction fragment length polymorphisms (RFLP) in *fliC* using the restriction enzyme *Rsa*I. A wide variety of *fliC* restriction fragment patterns was reported among isolates of 53 different flagellar antigen groups; the majority of the RFLP patterns observed corresponded to a unique H antigen group. Limited numbers of RFLP patterns were observed among members of some H groups, suggesting that the sequence of *fliC* within certain H groups is fairly well conserved. Four patterns were observed among strains expressing the H7 antigen. Interestingly, the same pattern was detected among all *E. coli* strains of serotype O157:H7 and 16 of 18 of serotype O55:H7, reflecting the common lineage of these strains observed by multilocus enzyme typing (Whittam et al., 1993). Nevertheless, sequencing of a total of 20 H7 *fliC* genes, representing 10 different serotypes (Reid et al., 1999; Wang et al., 2000), revealed a notable polymorphism in the *fliC* gene and identified 10 sequences with differences ranging from 0.06% to 3.12%. Recently, a collection of reference strains representing 48 H types was resolved into 62 patterns (F types) using *Hha*I restriction of the *fliC* gene (Machado et al., 2000). A single F type was associated with each of 39 H types and more than one

F type was associated with the other nine H types. Antigenically related H12 and H45 gave a single F type. The determination of *Hha*I-*fliC* F types could allow deduction of all H types and subdivision of some of these. The two above-mentioned molecular typing methods hold promise of a rapid, more refined and specific typing scheme for the H antigens, which may also be helpful in determining phylogenetic relatedness between different clones of *E. coli*. Furthermore, molecular typing has the advantage of allowing typing of nonmotile strains or of strains that do not (sufficiently) express the immunoreactive H antigen, and is likely to expand the present number of significantly different H types. The observed polymorphism in a single determinant, such as the H7 *fliC* gene, stresses the importance of solid and extensive validation of molecular typing with reference to the existing serotyping scheme, and calls for caution in the interpretation of patterns obtained by DNA fingerprinting methods.

Serotyping Subdivision of *E. coli* can be carried out in many ways, but serotyping remains one of the most useful ways to subdivide the species on a global basis. This typing method is based on the many antigenic differences found in structures on the bacterial surface. A serotype is recorded in the following way: O18ac:K1:H7 or O111:H2 (the latter antigenic formula indicates that K antigens are not present in the strain). MR fimbriae, which are present only in some, often pathogenic, serotypes, can also be used for the serological characterization (Ørskov et al., 1977, 1980b; Ørskov and Ørskov, 1990) in which case the complete serotype is recorded as O4:K3:H5; F13 or O147:H19; F4ac. Serotyping procedures are described in Gross and Rowe (1985) and Ørskov and Ørskov (1984a).

Even though complete serotyping involving the many known O, K, H, and F antigens has been carried out in only a very few laboratories, it is well known that the existing number of serotypes is very high.

Antibiotic or drug sensitivity Like other Gram-negative bacteria, *E. coli* is intrinsically resistant to hydrophobic antibiotics, such as macrolides, novobiocins, rifamycins, actinomycin D, and fusidic acid (Nikaido, 1996). The structure of the outer membrane of *E. coli* and its role in mediating intrinsic resistance to these molecules was reviewed by Nikaido (1996). Resistance to these compounds is attributed, in part, to the low permeability of the outer membrane bilayer to lipophilic solutes; however, active efflux mechanisms may have a synergistic effect on resistance in certain cases (Nikaido, 1996).

Acquired resistance to aminoglycosides, beta-lactams, chloramphenicol, macrolides, sulfonamides, tetracycline, and trimethoprim has been described for *E. coli* strains (reviewed by Quintiliani and Courvalin, 1995). Acquired resistance can develop by four distinct mechanisms: alteration of the target site, enzymatic detoxification of the antibiotic, decreased drug accumulation, and bypass of an antibiotic-sensitive step. The first three mechanisms may be mediated by chromosomal mutations or the acquisition of plasmids carrying resistance genes. The fourth mechanism is primarily attributable to the horizontal transfer of antibiotic resistance genes on a plasmid or transposon. The biochemical mechanisms and genetic basis of acquired resistance to antimicrobial agents of clinical importance was discussed by Quintiliani and Courvalin (1995). Genetic methods for the detection of antibacterial resistance genes were reviewed by Tenover et al. (1995).

Pathogenicity *E. coli* is a natural and essential part of the bacterial flora in the gut of humans and animals. Most *E. coli* strains are nonpathogenic and reside harmlessly in the colon; however, certain serotypes or clones play an important role in both intestinal and extraintestinal diseases. The diverse pathogenesis of this bacterium in apparently healthy individuals is largely attributable to its possession of a variety of specific virulence factors. In hosts with compromised defenses, *E. coli* can also be an excellent opportunistic pathogen.

E. coli IN HUMAN INTESTINAL DISEASES *E. coli* strains isolated from intestinal diseases have been grouped into at least six different main categories based on epidemiological evidence, phenotypic traits, clinical features of the disease they produce, and specific virulence factors. The currently recognized categories of diarrheagenic *E. coli* include enteropathogenic *E. coli* (EPEC) (actually a subgroup of attaching and effacing *E. coli* (A/EEC) defined as *eae* positive *E. coli* belonging to both the classical EPEC serotypes and nonclassical EPEC serotypes), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffusely adherent *E. coli* (DAEC), and Shiga toxin-producing *E. coli* (STEC), which are also referred to as Vero cytotoxin-producing *E. coli* (VTEC). These categories are reviewed below with emphasis on their virulence factors.

ENTEROPATHOGENIC *E. coli* (EPEC) Enteropathogenic *E. coli* was the first category of diarrheagenic *E. coli* to be recognized. The term *enteropathogenic E. coli* (EPEC) was originally used to refer to strains belonging to a limited number of O groups that were epidemiologically associated with infantile diarrhea (Neter et al., 1955). This rather imprecise definition, which allowed for the inclusion of a heterogeneous group of pathogens, was used for decades and became increasingly problematic as groups of *E. coli* that could produce diarrheal disease by the production of enterotoxins or invasion of intestinal epithelial cells were recognized. The confusion generated by the discovery of new pathogenic groups of *E. coli* and the findings that EPEC strains, which lacked the virulence properties of these newly recognized groups, caused disease in adult volunteers (Levine et al., 1978) prompted researchers in 1982 to define EPEC as "diarrheagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not yet been proven to be related to either heat-labile enterotoxins or heat-stable enterotoxins or *Shigella*-like invasiveness" (Edelman and Levine, 1983). As more was learned about the strains associated with infant diarrhea, the definition was refined to include only certain O:H serotypes associated with illness. Table BXII.γ.199 lists some of the O:H serotypes that have been regarded for many years as EPEC. Since 1982, advances in our understanding of the molecular aspects of EPEC pathogenesis have allowed researchers to move beyond the serologic markers that correlate with disease to develop a definition based on pathogenic characteristics. A definition adopted in 1995 identified the most important characteristics of EPEC as its ability to cause attaching and effacing (A/E) histopathology and its inability to produce Shiga toxins (Kaper, 1996). The pathogenesis of EPEC is highlighted below and has been reviewed in detail by Nataro and Kaper (1998) and Williams et al. (1997).

The first advance in understanding the pathogenesis of EPEC infection was the discovery that EPEC strains adhere to HEp-2 cells in cell culture (Cravioto et al., 1979) in a distinctive pattern termed localized adherence (LA) (Scaletsky et al., 1984). Expression of the LA phenotype in EPEC requires a plasmid re-

TABLE BXII.γ.199. O:H serotypes regarded as classical and newly recognized EPEC O:H serotypes[a,b]

O group	H antigen[c]	Comments
O26	H⁻; H11	O26:H⁻ and O26:H11 may also be STEC/VTEC[d] (Levine et al., 1987; Scotland et al., 1990; Bitzan et al., 1991)
O55	H⁻; H6; H7	O55:H7, H10 and H⁻ may also be STEC/VTEC (Dorn et al., 1989)
O86	H⁻; H8; H34	O86:H⁻ may also be EAggEC (Albert et al., 1993b; Tsukamoto and Takeda, 1993; Schmidt et al., 1995b; Smith et al., 1997b)
		O86:H8 is a new *eae*- and *bfpA*-positive type isolated in Denmark (Scheutz, unpublished data)
O88	H⁻; H25	(Tsukamoto et al., 1992)
O103	H2	New EPEC type
O111	H⁻; H2; H7; H12	O111:H⁻ may also be STEC/VTEC (Dorn et al., 1989; Bitzan et al., 1991; Caprioli et al., 1994; Cameron et al., 1995; Allerberger et al., 1996) or EAggEC (Scotland et al., 1991, 1994; Tsukamoto and Takeda, 1993; Chan et al., 1994; Schmidt et al., 1995b; Monteiro-Neto et al., 1997; Morabito et al., 1998)
O114	H⁻; H2	
O119	H⁻; H2; H6	
O125ac	H⁻; H6	O125 may also be EAggEC (Tsukamoto and Takeda, 1993; do Valle et al., 1997; Smith et al., 1997b)
O126	H⁻; H2; H21; H27	
O127	H⁻; H6; H21; H40	
O128ab	H⁻; H2; H7; H12	O128:H2 may also be STEC/VTEC (Beutin et al., 1993a)
O142	H⁻; H6; H34	
O145	H⁻; H45	New EPEC type
O157	H⁻; H8; H16; H45	New EPEC types
O158	H⁻; H23	

[a]Data from Cravioto et al. (1979), Levine and Edelman (1984), Levine et al. (1985), Scaletsky et al., 1985,Robins-Browne (1987), Gomes et al. (1989b), Knutton et al. (1989, 1991), Scotland et al. (1989, 1992, 1996), Ørskov and Ørskov (1992), Donnenberg (1995).

[b]O18:H⁻, H7, H14; O26:H34 and O44:H34 have also been listed but only in Knutton et al. (1991). O18 strains are probably not EPEC (Knutton et al., 1989; Ørskov and Ørskov, 1985). O44:H18 is now considered to belong to the group of enteroaggregative *E. coli* (Smith et al., 1994).

[c]Nonmotile strains of *E. coli* are regarded as descendants of motile strains that have lost their motility by mutation(s). Their original H antigen was often deduced from comparison of biochemical reactions (Kauffmann and Dupont, 1950; Staley et al., 1969).

[d]Abbreviations: EPEC, enteropathogenic *E. coli*; STEC/VTEC, Shiga toxin-producing *E. coli* /Vero cytotoxin-producing *E. coli*; EAggEC, enteroaggregative *E. coli*.

ferred to as the EPEC adherence factor (EAF) plasmid (Baldini et al., 1983). A 1-kb DNA probe originally thought to encode the EPEC adherence factor (EAF) necessary for LA was cloned from this plasmid (Nataro et al., 1985a) and has been used extensively as a marker to study the prevalence of EPEC infections (Nataro et al., 1985a; Echeverria et al., 1987, 1991; Gomes et al., 1989a, b; Moyenuddin et al., 1989; Senerwa et al., 1989; Cravioto et al., 1991; Kain et al., 1991; Strockbine et al., 1992; Begaud et al., 1993). EAF plasmids have been found in many EPEC serotypes and range in size from 26 to 76 MDa (Scotland et al., 1989) but typically are 50–70 MDa. Evidence supporting a role for the EAF plasmid in pathogenesis was provided by feeding studies showing that volunteers ingesting a plasmid-cured EPEC strain developed less diarrhea than those ingesting the plasmid-containing parental strain (Levine et al., 1985). Genes at two loci are necessary for expression of the LA phenotype: a cluster of 14 genes on the EAF plasmid involved in biogenesis of the bundle forming pilus (BFP), a type-IV pilus, which includes genes encoding bundlin (*bfpA*), the major structural subunit of the type-IV pilus, a prepilin peptidase, which processes pre-bundlin to its mature form, and 12 other proteins, and *dsbA* on the chromosome (Donnenberg et al., 1997).

A hallmark of the histopathology of EPEC infections is the presence of attaching and effacing (A/E) lesions in the intestinal tract. On electron micrographs of jejunal biopsies from children infected with EPEC, the bacteria are seen intimately attached to the epithelial cells on cup-like pedestals composed of depolymerized cytoskeletal proteins (Knutton et al., 1987). Microvilli are disrupted as a result of the cytoskeletal rearrangements and effaced by vesiculation (Fig. BXII.γ.192). The intimate attachment of EPEC is mediated by a protein known as intimin, which is a 94-kDa outer membrane protein encoded by the *eae* gene (*E. coli* attaching and effacing) (Jerse and Kaper, 1991). A 1-kb fragment of the *eae* gene referred to as CVD434 has been cloned (Jerse et al., 1990) and used to screen for attaching and effacing *E. coli* A/EEC (Bokete et al., 1997), and to characterize enteropathogenic *E. coli* (Scotland et al., 1996). Intimins belong to a growing family of proteins. In human EPEC strains, intimins α, β, γ, λ (δ not now used, although it is in the literature because it is a variant of the ε variant), and ε derivatives have been found to be serotype-specific and exhibit specific different binding affinities (Agin and Wolf, 1997). Intimins are also found in rabbit EPEC RDEC-1 strains, A/EEC strains from dogs (Beaudry et al., 1996), pigs (Zhu et al., 1995), and in *C. freundii* (Int_{CF}) and *Citrobacter rodentium* (Int_{CR}). Five strains initially identified as *Hafnia alvei* were reported to contain the *eae* gene (Albert et al., 1992), and a protein referred to as Int_{HA} has been characterized and compared to the above intimins. However, the strains are actually unusual biotypes belonging to the genus *Escherichia* (Janda et al., 1999), most likely *E. coli*. The *eae* gene is only one of many genes located on a pathogenicity island (PAI) known as the locus of enterocyte effacement (LEE) (McDaniel et al., 1995).

EPEC strains secrete at least four proteins, Esps for EPEC secreted proteins, encoded by LEE. EspA, B, D, and Tir proteins are secreted via the type III apparatus and are required for attaching and effacing activity. Protein secretion, the transcription

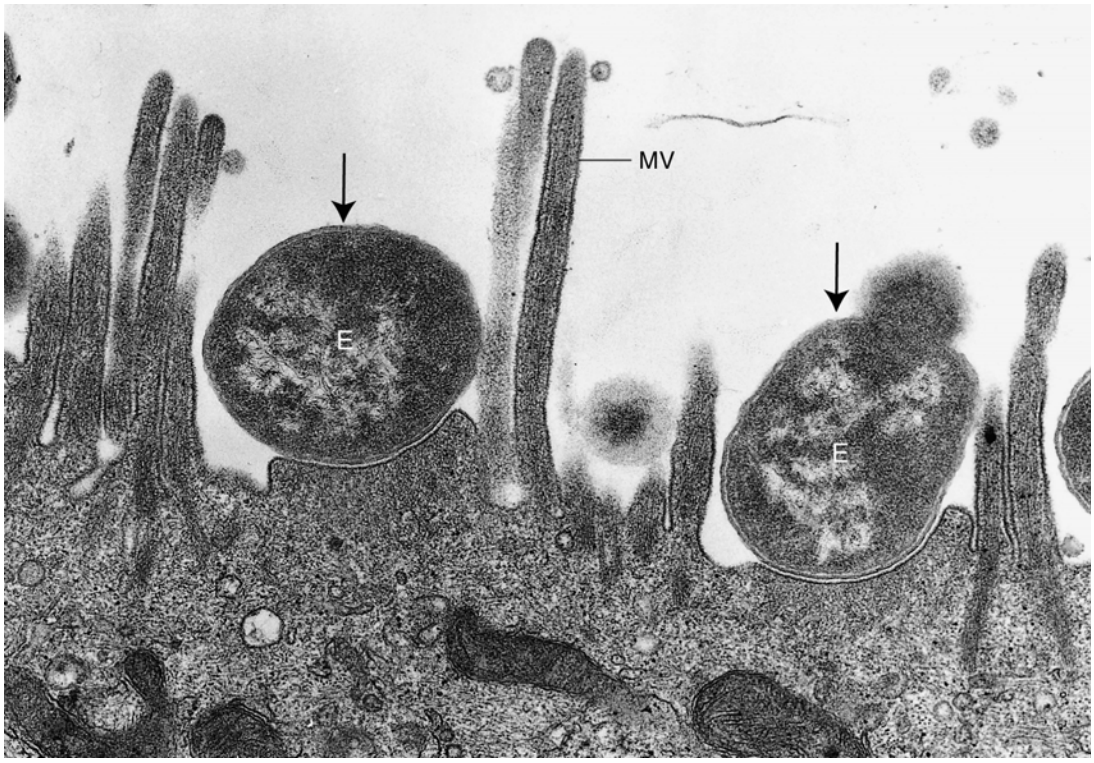


FIGURE BXII.γ.192. EM of cultured human intestinal mucosa infected with EPEC strain E2348 (*E*) for 8 h. Bacteria are seen intimately attached to the epithelial cells on cup-like pedestals composed of depolymerized short filament cytoskeletal proteins (*arrows*). Brush border microvilli (*MV*) are disrupted as a result of the cytoskeletal rearrangements and effaced by vesiculation. (Reproduced with permission from S. Knutton et al., Infection and Immunity *55:* 69–77, 1987, ©American Society for Microbiology.)

of the intimin gene (*eae*), and the synthesis of the bundle-forming pilus are all affected by growth conditions (Haigh et al., 1995; Jarvis et al., 1995; Kenny and Finlay, 1995) and a plasmid-encoded regulatory region. The plasmid locus is either referred to as the *per* (plasmid-encoded regulator) genes (*perABCD*) (Gómez-Duarte and Kaper, 1995) or as an integrated part of the bundle-forming pilus (*bfp*) operon, genes *bfpT*, *V*, and *W* (Tobe et al., 1996).

EPEC interferes with normal pathways of signal transduction in epithelial cells. EPEC will induce tyrosine phosphorylation; phosphorylation of myosin light chain, vinculin, and α-actinin; *in vitro* release of intracellular calcium; phospholipase C activity, resulting in elevated levels of inositol phosphates and diacylglycerol, in turn activating protein kinase C; reactions that induce host cell proteins to initiate cytoskeletal rearrangement; and bacterial uptake (Rosenshine et al., 1992; Manjarrez-Hernandez et al., 1996).

EPEC is not invasive in the same way as *Shigella*, *Yersinia*, and *Salmonella* (Geyid et al., 1996), although some EPEC strains may exhibit *in vitro* invasion of epithelial cells at levels comparable to those seen in EIEC (Donnenberg et al., 1989; Pelayo et al., 1999). A 4.5-kb fragment from a large so-called EPEC plasmid of an O111:H$^-$ strain negative for both EAF and *eae* will confer epithelial cell invasivity and the attaching and effacing ability on a noninvasive laboratory strain (Fletcher et al., 1990, 1992). Only a smaller proportion of EPEC strains hybridize with the cloned fragment.

Of the EPEC O:H serotypes shown in Table BXII.γ.199, only 16 of the possible 175 recognized O groups are represented and only a few H antigens, e.g., H2, H6, H7, H12, H21, and H34, occur among several of these 16 O groups. The more recently described serotypes possessing EPEC-associated virulence markers have been added to the list. Some of these are O86:H8, O88:H25, O103:H2, O127:H40, O142:H34, O145:H45, O157:H8, O157:H16, and O157:H45.

Enterotoxigenic *E. coli* (ETEC) Enterotoxigenic *E. coli* strains are important causes of diarrhea in both humans and domestic animals. ETEC strains do not invade epithelial cells but produce one or more enterotoxins that are either heat-labile (LT), which is closely related to cholera toxin, or heat-stable (ST) (Cohen and Giannella, 1995). LT-I and ST are plasmid-encoded (LT-II is chromosomally encoded) and found alone or together, and are often associated with a limited number of O:K:H serotypes and O groups. Table BXII.γ.200 shows some of the most common serotypes from humans. These enterotoxins cause intestinal secretion either by activation of guanylate (ST) or adenylate (LT) cyclase and are subdivided based on their biological activities, receptors, and chemical and antigenic properties. STh and STp are used to indicate strains of human or porcine origin. The gene encoding another heat-stable enterotoxin called enteroaggregative heat-stable toxin 1 (EAST1), originally thought to be produced by EAggEC only, may also be present in addition to the STa gene. The different variants of enterotoxins are summarized in Table BXII.γ.201.

There is host specificity among ETEC strains causing diarrhea in humans or different species of domestic animals. This is mainly due to the specific recognition between bacterial colonization factors and the epithelial receptors during host-parasite inter-

TABLE BXII.γ.200. O:(K):H serotypes of human ETEC

O group	(K):H antigen	
O6	H^-	K15:H16
O7	H^-	H18
O8	K47:H^-	K25:H9; K40:H9; H10; K87:H19
O9	H^-	K9; K84:H2
O11		H27
O15		H11; H15; H45
O17		K23:H45; H18
O20	H^-	H30
O21		H21
O25	H^-	K7:H42; H16
O27		H7; H20; H27
O29		H?
O48		H26
O55		H7
O56	H^-	
O63		H12; H30
O64	H^-	
O65		H12
O71		H36
O73		H45
O77		H45
O78	H^-	K2; H11; H12
O85		H7
O86		H2
O88		H25
O105		H?
O114	H^-	H21
O115	H^-	H21; H40; H51
O119		H6
O126	H^-	H9; H12
O128ac		H7; H12; H19; H21
O133		H16
O138		K81
O139		H28
O141	H^-	H4
O147		H?
O148		H28
O149		H4; H10; H19
O153		H10
O159	H^-	H4; H5; H12; H20; H21; H27; H34; H37
O166		H27
O167		H5
O?		H2; H10; H28; K39:H32

TABLE BXII.γ.201. Variants of enterotoxins found in ETEC strains[a]

Toxin type	Subtypes		Comments
LT: Heat-labile enterotoxins			
LT-I	LTp (LTp-I)	LTh (LTh-I)	Associated with disease in both humans and animals
LT-II	LT-IIa	LT-IIb	No specific association with disease. Rare in human isolates
ST: Heat-stable enterotoxins			
STa (STI)	STp (STIa)	STh (STIb)	Produced by ETEC and several other Gram-negative bacteria. In ETEC, ST is often found together with the genes for EAST1.
STb (STII)			Induces histological damage in the intestinal epithelium. Most often found in porcine ETEC.

[a]Suffixes commonly used are: h, human variant; p, porcine variant.

action. Most of the fimbriae found in ETEC are typically MR fimbriae. In human ETEC strains at least 21 different surface structures called CS, for coli surface antigens, or CFA, for colonization factor antigens, usually plasmid encoded, have been described (Gaastra and Svennerholm, 1996). In animals, the most common fimbriae are F4 (which was originally described as K88), F5 (originally named K99), F6 (originally named 987P), F17, F41, F42, CS31A, F141, and F165. These fimbriae are found in enterotoxigenic strains from newborn piglets, pigs, lambs, and newborn calves. A close association between the EAST1 toxin and CS31A, which is related to F4, among pathogenic bovine *E. coli* has been suggested (Bertin et al., 1998).

More recently, the fimbrial F18 antigen (provisionally designated F107, 2134P, or 8813) has been found in both porcine ETEC and STEC/VTEC strains. It is characteristic that the fimbriae usually are found in a limited number of serotypes. The antigenic variants of F18 fimbriae (F18ab and F18ac) are biologically distinct. F18ab fimbriae are expressed poorly both *in vitro* and *in vivo* and are frequently linked with the production of Stx2e/VT2e and O group O139, while F18ac are more efficiently expressed *in vitro* and *in vivo* and most often are linked with enterotoxin (STa, STb) production, and O groups O141 and O157.

Enteroinvasive *E. coli* (EIEC) Enteroinvasive *E. coli* are very similar to *Shigella*. Like *Shigella*, they are capable of invading and multiplying in the intestinal epithelial cells of the distal large bowel in humans. Approximately two thirds of EIEC strains are lactose negative and virtually all are lysine negative. Multiple chromosomal and plasmid genes are associated with virulence (Acheson and Keusch, 1995). EIEC are restricted to a very limited number of serotypes, most of which are nonmotile (Table BXII.γ.202).

Enteroaggregative *E. coli* (EAggEC) Enteroaggregative *E. coli* are characterized by a distinct aggregative adherence (AA) pattern to HEp-2 cells *in vitro* first described by Nataro et al. (1987). This pattern is distinguished by the prominent autoagglutination of bacterial cells to each other, to the surface of the HEp-2 cells, as well as to the glass cover slip in a characteristic layering best described as a "stacked brick" configuration. The AA pattern is plasmid-mediated (Nataro et al., 1985b) and was suspected to be a putative agent of diarrheal disease as early as 1988 (Vial et al., 1988). Volunteer studies with EAggEC have indicated that certain types will cause diarrhea and other enteric symptoms including borborygmia and cramps (summarized in

TABLE BXII.γ.202. O:H serotypes of EIEC

O group	H antigen
O28ac	H^-
O29	H^-
O112ac	H^-
O115	H^-
O121	H^-
O124	H^-; H7; H30; H32
O135	H^-
O136	H^-
O143	H^-
O144	H^-; H25
O152	H^-
O159	H2
O164	H^-
O167	H^-; H4; H5
O173	H^-

Nataro, 1995), and epidemiological studies have implicated EAggEC as a cause of travelers' diarrhea (Brook et al., 1994; Scotland et al., 1994). Only about half of the case-control studies that have been carried out find significantly higher isolation rates in cases than in controls (summarized by Law and Chart, 1998). Most recently, EAggEC have been associated with acute and chronic diarrhea and abdominal colic in young children in Germany (Huppertz et al., 1997), and with four outbreaks of gastroenteritis in the UK (Smith et al., 1997b). A DNA probe, referred to as CVD432, has been cloned from the plasmid of strain O42, serotype O44:H18 (formerly an EPEC O:H serotype) and used to identify EAggEC (Baudry et al., 1990). A plasmid encoded flexible, bundle-forming fimbrial structure, designated aggregative adherence fimbria I (AAF/I), is encoded by two regions (Nataro et al., 1992, 1993). Region 2 encodes a transcriptional activator of the AraC family of DNA-binding proteins (Nataro et al., 1993). At least one toxin similar to the heat-stable toxin of ETEC, ST designated EAST1, has been identified in an EAggEC strain (Savarino et al., 1991, 1993). The gene encoding EAST1 has been shown to be broadly distributed among diarrheagenic *E. coli*: 100% of 75 O157:H7 STEC/VTEC, 41% of 227 EAggEC, 41% of 149 ETEC, 22% of 65 EPEC, and 38% of 47 *E. coli* from asymptomatic children hybridized with the EAST1 DNA probe (SS126) (Savarino et al., 1996). Despite the common occurrence of the EAST1 gene in many diarrheagenic *E. coli* groups, outbreaks had not been attributed to EAST1-only-producing *E. coli* until recently, when an outbreak in Japan was associated with an O166:H? strain positive for the EAST1 gene (Nishikawa et al., 1999). In Spain, a case-control study suggested that EAST-1 positive *E. coli* strains are associated with diarrheal diseases in Spanish children, whereas EAggEC strains are not (Vila et al., 1998). The significance of these findings remains to be established. Many serotypes have been observed and some of the most commonly reported serotypes are listed in Table BXII.γ.203.

Diffusely adherent *E. coli* (DAEC) Diffusely adherent *E. coli* are defined by the presence of the diffuse adherence (DA) pattern of *E. coli* strains to HEp-2 cells (Scaletsky et al., 1984; Nataro et al., 1985b). A surface fimbriae designated F1845 confers the DA phenotype, and a DNA probe has been cloned (Bilge et al., 1989). Another adhesin (designated AIDA-I) has also been associated with DA of *E. coli* of serotype O126:H27 (Benz and Schmidt, 1989). The role of DAEC in diarrhea is unclear.

TABLE BXII.γ.203. O:H serotypes of the most frequently reported enteroaggregative *E. coli* (EAggEC)[a]

O group	H antigen	References listing these serotypes as EAggEC
O3	H2	Albert et al. (1993b)
O15	H18	Vial et al., 1988, Albert et al. (1993b), Tsukamoto and Takeda (1993), Scotland et al. (1994)
O44	H$^-$; H18	Scotland et al. (1991, 1994), Tsukamoto and Takeda (1993), Smith et al. (1994), Schmidt et al. (1995b)
O86	H$^-$	Albert et al. (1993b), Tsukamoto and Takeda (1993), Schmidt et al. (1995b), Smith et al. (1997b)
O111	H12; H21	Scotland et al. (1991, 1994), Tsukamoto and Takeda (1993), Chan et al. (1994), Schmidt et al. (1995b), Monteiro-Neto et al. (1997), Morabito et al. (1998)
O125	H9; H21	Tsukamoto and Takeda (1993), do Valle et al. (1997), Smith et al. (1997b)

[a]Many other serotypes of EAggEC have been published by several authors.

Shiga toxin-producing *E. coli* (STEC) or Vero cytotoxin-producing *E. coli* (VTEC) Shiga toxin-producing *E. coli* or Vero cytotoxin-producing *E. coli* strains are characterized by their ability to produce either one or both of at least two antigenically distinct, usually bacteriophage-mediated cytotoxins referred to as Stx1 or VT1 (first described as Shiga-like toxin I, SLTI) and Stx2 or VT2 (first described as Shiga-like toxin II, SLTII). Whereas STEC/VTEC refers to all *E. coli* strains that produce Stx/VT in culture supernatants (Konowalchuk et al., 1977, 1978), the term *enterohemorrhagic E. coli* (EHEC) has been used to refer to strains that have the same clinical and pathogenic features associated with the prototype organism *E. coli* O157:H7 (Levine, 1987). In practice, EHEC is used to describe a subgroup of STEC/VTEC that causes hemorrhagic colitis.

Shiga toxins or Vero cytotoxins belong to the Shiga toxin family, comprises the following members: Shiga toxin, which is produced by *Shigella dysenteriae* type 1, Stx1/VT1, STx2/VT2, and Stx2/VT2 variants (Stxv/VT2v). Stx/VT genes, products, and synonyms are summarized in Table BXII.γ.204. Stx2e/VT2e, one of the variants, is produced by STEC/VTEC strains causing edema disease (Marques et al., 1987), a usually fatal disease, in weanling pigs and referred to as Stx2e/VT2e. Unlike all other Stxs/VTs, this is not cytotoxic to HeLa cells and binds to a different receptor, Gb4. The functional receptor for human Stx/VT is the glycolipid globotriosyl ceramide Gb3 (galactose-α-(1-4)-galactose-β-(1-4)-glucose ceramide) (Lingwood et al., 1987; Waddell et al., 1988) found in human renal endothelial cells (Obrig et al., 1993).

Stxs/VTs inhibit protein synthesis by depurination of adenine in 28S rRNA (*N*-glycosidases), thus inhibiting the elongation factor 1 (EF-1)-dependent aminoacyl-tRNA binding to 60S ribosomal subunits (Endo et al., 1988). In Vero cells the result is cell death by apoptosis (Inward et al., 1995).

Nomenclature of Shiga toxins/verocytotoxins The recognition and investigation of cytotoxin-producing *E. coli* infections by several groups has resulted in the use of differing systems of nomenclatures for the toxins produced by these bacteria. In 1994, O'Brien et al. (1994) developed a proposal for rationalizing the nomenclature of the *E. coli* cytotoxins. In their proposal, they recommended guidelines for classifying and designating members of the toxin family and stated the toxins shall be referred to by two alternate but interchangeable names, Shiga-like toxins (SLT) and Vero cytotoxins (VT). Two years later, a proposal to simplify the Shiga-like toxin nomenclature was published by Calderwood et al. (1996). In this proposal, the word "like" was omitted and the toxins and genes were renamed to reflect their relationship to Shiga toxin, the prototype toxin for the family. To avoid confusion in the literature, it was suggested that cross-reference to existing VT nomenclature could be used. While the omission of the word "like" has received general acceptance in the scientific community, strong arguments for maintaining the existing phenotype nomenclatures for *E. coli* cytotoxins were immediately put forward (Karmali et al., 1996), and the two systems of nomenclature are still being widely used. Toxins included in the Shiga toxin/Verocytotoxin family share the following properties: DNA sequence homology and operon structure (A subunit gene immediately upstream of the B subunit gene); polypeptide subunit structure (five B subunits to one A subunit in the mature holotoxin); enzymatic activity (*N*-glycosidases); binding to specific glycolipid receptors and biological properties, including enterotoxicity in ligated rabbit ileal loops;

TABLE BXII.γ.204. Designation of *stx*/*vtx* genes, their products (Stx/VT)[a], and their previous designations

Toxin type[b]	Toxin gene	Toxin	Prototype organisms (reference)	Serotype of prototype organism	Previous designation or synonym for toxin gene	Previous designation or synonym for toxin
1	stx_1/*vtx*1	Stx1/VT1	EDL933 (O'Brien et al., 1984)	O157:H7	*slt*-I	SLT-I
			H19 (Konowalchuk et al., 1977)	O26:H11		
			H30 (Konowalchuk et al., 1977)	O26:H11		
	$stx_{1\text{-}O103}$/*vtx*1-O103	Stx1-O103/VT1-O103	PMK1 (Mariani-Kurkdjian et al., 1993)	O103:H2		
	$stx_{1\text{-}O111\text{-}PH}$/*vtx*1-O111-PH	Stx1-O111-PH /VT1-O111-PH	PH (Paton et al., 1993a)	O111:H$^-$	*slt*I/PH	Slt-I/PH
	$stx_{1\text{-}OX3}$/*vtx*1-OX3	Stx1-OX3/VT1-OX3	131/3 (Paton et al., 1995a)	OX3:H8	*slt*I/OX3	Slt-I/OX3
	$stx_{1\text{-}O48}$/*vtx*1-O48	Stx1-O48/VT1-O48	94C (Paton et al., 1995a)	O48:H21	*slt*I/O48	Slt-I/O48
	$stx_{1\text{-}O111\text{-}CB168}$/*vtx*1-O111-CB168	Stx1-O111-CB168/VT1-O111-CB168	CB168 (Paton et al., 1995a)	O111:H$^-$	*slt*I/CB	Slt-I/CB
2	stx_2/*vtx*2	Stx2/VT2	EDL933 (O'Brien et al., 1984)	O157:H7	*slt*-II	SLT-II
2c	stx_{2c}/*vtx*2c	Stx2c/VT2c	E32511 (Schmitt et al., 1991)	O157:H$^-$		Stx2v/VT2v
					slt-IIc	SLT-IIc
	$stx_{2\text{-}O22}$/*vtx*2-O22	Stx2-O22/VT2-O22	KY-O19 (Lin et al., 1993a)	O22:H$^-$		VT2v(pKTN1054)
	$stx_{2\text{-}O157\text{-}TK\text{-}51}$/*vtx*2-O157-TK-51	Stx2-O157-TK-51/VT2-O157-TK-51	TK-51 (Lin et al., 1993a)	O157:H7		VT2v(pKTN1050)
	$stx_{2\text{-}OX3/a\text{-}031}$/*vtx*2-OX3/a-031	Stx2-OX3/a-031/VT2-OX3/a-031	031 (Paton et al., 1992)	OX3:H21	$stx_{2vOX392}$	SLT-II/OX3/VT2d-OX3[d]; SLT-II/OX3a
	$stx_{2\text{-}OX3/b\text{-}031}$/*vtx*2-OX3/b-031	Stx2-OX3/b-031/VT2-OX3/b-031	031 (Paton et al., 1993b)	OX3:H21	$stx_{2vOX393}$	SLT-II/OX3/2/VT2d-OX3/2[c]; SLT-II/OX3b
	$stx_{2\text{-}O48}$/*vtx*2-O48	Stx2-O48/VT2-O48	94C (Paton et al., 1995b)	O48:H21		SLT-II/O48
	$stx_{2\text{-}O111\text{-}PH}$/*vtx*2-O111-PH	Stx2-O111-PH /VT2-O111-PH	CB168 (Paton et al., 1995b)	O111:H$^-$	stx_{2vO111}	SLT-II/O111/VT2d-O111[c]
	$stx_{2\text{-}O118}$/*vtx*2-O118	Stx2-O118/VT2-O118[d]	EH250 (Piérard et al., 1998)	O118:H12		Stx2d/VT2d-Ount
	$stx_{2\text{-}O6}$/*vtx*2-O6	Stx2-O6/VT2-O6	NV206 (Bertin et al., 2001)	O6:H10	$stx_{2\text{-}NV206}$	Stx2-NV206
2d[d]	stx_{2d}/*vtx*2d	Stx2d/VT2d	B2F1 (Ito et al., 1990)	O91:H21		SLT-IIvh
	stx_{2d1}/*vtx*2d1	Stx2d1/VT2d1	B2F1 (Ito et al., 1990)	O91:H21	*stx*2vha/*vtx*2vha	Stx2vha/VT2vha
					*stx*2ha	Stx2vh-a/VT2vh-a
						VT2v-a
						SLT-IIvha
	stx_{2d2}/*vtx*2d2	Stx2d2/VT2d2	B2F1 (Ito et al., 1990)	O91:H21	*stx*2vhb/*vtx*2vhb	Stx2vhb/VT2vhb
					*stx*2hb	
						Stx2vh-b/VT2vh-b
						VT2v-b
						SLT-IIvhb
2e	stx_{2e}/*vtx*2e	Stx2e/VT2e	412 (Gyles et al., 1988)	O139:K12:H1	*slt*-IIv	SLT-IIv
			S1191 (Weinstein et al., 1988)	O139:H1	*slt*-IIva	SLT-IIva[e]
					slt-IIe	SLTIIe/VTe
						VT2vp
						VT2vp1
2f[f]	stx_{2f}/*vtx*2f	Stx2f/VT2f	H.I.8 (Gannon et al., 1990)	O128:H2	stx_{2ev}/*vtx*2ev	Stx2ev/VT2ev
			T4/97 (Schmidt et al., 2000)	O128:H2		Stx2vp2/VT2vp2
						VTev
					slt-IIvhc	SLTIIvhc
					slt-IId[d]	SLT-IId/VT2d[d]

[a]Data compiled from references Bastian et al. (1998), Bertin et al. (2001), Calderwood et al. (1996), Gannon et al. (1990), Ito et al. (1990), W.M. Johnson et al. (1991), Karmali et al. (1996), Kokai-Kun et al. (2000), Lin et al. (1993a, b), Takeda et al. (1993), and Tyler et al. (1991).

[b]Toxin types are defined according to antigenic variability, differences in toxicity for tissue culture cells and/or animals, their capacity to be activated by mouse elastase, and differences in DNA or amino acid sequences.

[c]There are several toxins suffixed by d in the literature: The Stx2d/VT2d toxins of O91:H21 (Melton-Celsa and O'Brien, 1998), the VT2d (= stx2-O group/ strain designation and/or year) variants by Paton et al. (1992, 1993a, 1995b), Piérard et al. (1998), and the SLT-IId/VT2d (= Stx2f/VT2f) toxin produced by strain H.I.8 (serotype O128:H2) as proposed by Gyles (1994). We support the use of the d designation for activatable Stx2/VT2 toxins as proposed by Melton-Celsa and O'Brien, (1998).

[d]Stx2-O118/VT2-O118 (formerly known as VT2d-Ount) is expected to be nonactivatable based on analysis of the nucleotide sequence (Denis Piérard and Angela Melton-Celsa; unpublished); the original strain has been retyped as O118:H12 (Lothar Beutin and Flemming Scheutz, unpublished).

[e]This designation has also been referred to for the Stx2f/VT2f toxin produced by strain H.I.8 (serotype O128:H2).

[f]The nucleotide sequence of the former stx_{2ev}/*vtx*2ev of strain H.I.8 (serotype O128:H2) is nearly identical to the recently published stx_{2f}/*vtx*2f found in strain T4/97 (serotype O128:H2) from feral pigeons (Schmidt et al., 2000). As its nucleotide sequence is distinctly different from those of the other Stx2/VT2 toxins and variants as well as Stx1/VT1, we support the proposal of renaming stx_{2ev}/*vtx*2ev as stx_{2f}/*vtx*2f.

neurotoxicity in mice; and cytotoxicity to receptor-expressing tissue culture cell lines, such as Vero and HeLa cell lines. Classification of the toxins into major toxin types, designated with Arabic numbers, is based on differences that result in no cross-neutralization by homologous polyclonal antisera and no DNA–DNA cross-hybridization of their genes under conditions of high stringency. Toxin subtypes, designated with letters added to the type name, share cross-hybridization of their genes under high stringency but show significant differences in biologic activity, including the capacity to be activated; serologic reactivity; or receptor binding. Table BXII.γ.204 summarizes the currently reported Shiga toxin/Verocytotoxin genes and toxins, prototype organisms, and their previous designations in the literature.

The distinction between toxin types 2c and 2d in Table BXII.γ.204 is based on differences in the A subunit determining whether the toxin is activatable (Stx2d/VT2d) or nonactivatable (Stx2c/VT2c). The other Stx2/VT2 subtypes described in the literature, which have not been tested for all properties, have been tentatively placed together with toxin type 2c, primarily based on similarities in nucleotide sequences that place them in a phylogenetically related Stx2/VT2 cluster including all variants. Based on their degree of overall nucleotide sequence similarity, Stx2/VT2 toxins fall into two phylogenetically distinct groups (Bastian et al., 1998; Piérard et al., 1998). Toxins in group 1, which include Stx2/VT2, Stx2c/VT2c (including variants Stx2-O22/VT2-O22, Stx2-O157-TK-51/VT2-O157-TK-51, Stx2-OX3/b-031/VT2-OX3/b-031, Stx2-O48/VT2-O48), and Stx2d/VT2d, share 99.1–99.2% and 95.9–98.5% nucleotide sequence similarity in their A and B subunits, respectively; while toxins in group 2, which include Stx2-OX3/a-031/VT2-OX3/a-031, Stx2-O111-PH/VT2-O111-PH, and Stx2-O118/VT2-O118, share 96.9–99.9 % and 99.6–100% nucleotide sequence similarity in their A and B subunits, respectively. The similarities of individual sequences between group 1 and group 2 are 93.4–96.0% for the A subunits and 86.2–89.3% for the B subunits. The extent of, and differences between, toxicity for tissue culture cells and/or animals and their capacity to be activated are not fully established for all the types. Further analysis against all the criteria necessary to allow definitive placement with appropriate other toxin types is required. In the absence of this information, suffixes have been added after the O group of the source organisms, and, when necessary, the original strain designation. Stx2/VT2 subtype toxins found in the same original strain are suffixed "/a", "/b" etc. as in Stx2-OX3/a-031/VT2-OX3/a-031 and Stx2-OX3/b-031/VT2-OX3/b-031.

For a brief presentation and practical use of the subtyping of Stx/VT genes with 4 Stx1/VT1 and 9 Stx2/VT2 oligonucleotide DNA probes, and 3 Stx1/VT1 and 8 Stx2/VT2 primer pairs, see Smith et al. (1993), Yamasaki et al. (1996), Piérard et al. (1997), and Bastian et al. (1998).

Like EPEC, some STEC/VTEC strains have been shown to cause attaching and effacing lesions *in vivo* (Hall et al., 1990), in animal models (Francis et al., 1986; Tzipori et al., 1986; Sherman et al., 1988), and *in vitro* (Knutton et al., 1989). Two separate groups have cloned, sequenced, and characterized the *eae* homologue from VTEC O157:H7 (Beebakhee et al., 1992; Yu and Kaper, 1992). Homology between EPEC and STEC/VTEC sequences was 86% and 83% at the nucleotide and amino acid levels, respectively (Yu and Kaper, 1992), and the STEC/VTEC *eae* sequence was 97% homologous to the EPEC *eae* gene for the first 2200 bp and 59% homologous over the last 800 bp (Beebakhee et al., 1992). Both *eae* sequences show 50% homology to the central region of the *Yersinia pseudotuberculosis inv* gene (Jerse et al., 1990; Beebakhee et al., 1992), and the predicted amino acid sequence of the STEC/VTEC *eae* gene share 31% identity and 51% similarity with the invasin molecule of *Yersinia pseudotuberculosis* (Yu and Kaper, 1992).

Serotype specific heterogeneity of the *eae* gene in STEC/VTEC strains O55:H7 or H^-, O111:H8 and O157:H7 or H^-, and in O groups O26, O103, and O157 has been demonstrated (Gannon et al., 1993; Louie et al., 1994).

Almost all STEC/VTEC O157:H7 strains harbor a large 60-65 MDa plasmid (Johnson et al., 1983), designated pO157, that plays a role in virulence (Karch et al., 1987) and a small plasmid of 6.6 kb found in O157:H7 STEC/VTEC strains appear to synthesize colicin D (Bradley et al., 1991). O26:H11 strains also possess at least one plasmid in the range of 55–70 MDa. Restriction enzyme patterns of plasmids from other O:H serotypes (including O5, O91, O103, O111, O121, and O127) show a notable similarity with the large plasmids in O157 and O26 strains (Levine et al., 1987). A 3.4-kb fragment from a large plasmid of O157:H7 (prototype EDL 933) has been cloned and used as a DNA probe (referred to as CVD419) to identify EHEC plasmids (Levine et al., 1987); i.e., large plasmids found in Verocytotoxin producing *E. coli* strains. DNA probing with gene probes defining the incompatibility group of plasmids indicates that the STEC/VTEC plasmids share an approximately 23-kb fragment with EPEC plasmids and that the large plasmids of both EPEC and STEC/VTEC constitute a family of transfer-deficient Inc F-IIA plasmids (Hales et al., 1992), while sequencing of pO157 reveals high homology to the orf1 of the RepFIB replicon (Schmidt et al., 1996a).

The large plasmid of O157 encodes the EHEC-hemolysin (Ehx), which is homologous to the *E. coli* α hemolysin (Schmidt et al., 1994, 1995a), and a novel catalase-peroxidase, KatP (Brunder et al., 1996). In contrast to α hemolysin, Ehx can be detected only on blood agar plates containing washed sheep erythrocytes. The zones of hemolysis on these plates are smaller and more turbid than those caused by α hemolysin and require overnight incubation before they become visible (Beutin, 1991; Beutin et al., 1988, 1989). A role for Ehx in the pathogenesis of diarrheal disease has not been demonstrated. Because α hemolysin-producing strains are uncommon in feces, these hemolysins serve as useful phenotypic markers for the detection of the majority of STEC/VTEC organisms.

The genes encoding the Ehx constitute a typical RTX (repeats in toxin) determinant, the Ehx-operon, with the gene order CABD (Schmidt et al., 1996a). The *ehxA* gene encodes the active protein, and *ehxB* and *ehxD* share high sequence homology with other RTX transport proteins (Schmidt et al., 1995a, 1996a). Like α hemolysin, the Ehx is a highly active cytolysin of the RTX family with a similar but not identical pore-forming capacity (Schmidt et al., 1996b). The Ehx plasmid DNA probe (CVD419) covers the *ehxA* and part of the *ehxB* gene (Schmidt et al., 1995a).

Two other enterohemolysins Ehly1 and Ehly2 have been described (Beutin et al., 1993b; Stroeher et al., 1993). Ehly1 is a 33-kDa cell-associated protein encoded by a bacteriophage, ΦC3888, found in O26:H11 STEC/VTEC. Ehly1 has no known sequence homology to any other DNA or protein sequence. The Ehly2 enterohemolysin is also encoded by a bacteriophage, ΦC3208, found in O26:H11. It is in part homologous to DNA of bacteriophageλ; but completely unrelated to Ehly1 (Beutin et al., 1993b).

Most information on the source and transmission of STEC/VTEC has been learned from outbreak investigations. Findings

from these investigations showed that most outbreaks are related to carriage of the organism in ruminants, especially cattle, which show no symptoms of disease. During the period from 1982 to 1993, at least 20 outbreaks of O157:H7 have been reported in the USA (summarized in Anonymous, 1994). These outbreaks have affected 1509 patients, resulting in the hospitalization of 346 patients, 86 cases of HUS, and 19 deaths. This increased dramatically in the following years with 13 outbreaks in 1993 and 30 outbreaks in 1994 (Armstrong et al., 1996). The largest multistate outbreak in the USA occurred in early 1993 with more than 700 illnesses and 4 deaths (Bell et al., 1994; Davis, 1994). It has been estimated that *E. coli* O157:H7 causes 73,000 illnesses annually in the United States and non-O157 STEC/VTEC, 37,000 illnesses; and that 91 deaths occur each year in the USA (Mead et al., 1999). In Canada, 15 outbreaks were reported in 1982–1987 with 242 cases, 24 cases of HUS, and 15 deaths (Karmali, 1989). The first recognized community outbreak of O157:H7 in Europe occurred in the UK in the summer of 1985 affecting at least 24 persons. Eleven patients were hospitalized and one died (Morgan et al., 1988). In England and Wales, O157:H7 was isolated from 39% of sporadic cases of hemorrhagic colitis (Smith et al., 1987) and 33% of sporadic HUS cases (Scotland et al., 1988). In an outbreak of HUS in the West Midlands, O157:H7 was isolated from 33% of cases (Taylor et al., 1986a; Willshaw et al., 2001). Subsequent outbreaks and sporadic cases in the UK have been reported (Salmon et al., 1989). Scotland has one of the highest rates of infection with O157 increasing from 1.37/100,000 of the population in 1989 (Thomas et al., 1996a) to 32.3/100,000 in 1996 (Reilly and Carter, 1997). The worst food poisoning outbreak with O157 STEC/VTEC in Scotland occurred in 1996 with 501 cases; 151 were hospitalized and 20 elderly died (Ahmed and Donaghy, 1998).

O157 STEC/VTEC has been isolated from outbreaks and from sporadic cases of diarrhea and HUS in many parts of the world: Canada, UK, Argentina, Germany, Central Europe, Chile, and Italy (summarized by Griffin, 1995; see also Chapters 2–9 in Kaper and O'Brien, 1998). Sakai City in Japan experienced the largest outbreak of O157 STEC/VTEC ever recorded in July 1996, which was part of several outbreaks that summer with an estimated number of a little less than 8,000 cases and 6 deaths (Infectious Disease Surveillance Center, Japan, 1997).

Other STEC/VTEC O:H serotypes have caused outbreaks of diarrhea and HUS: O111:H$^-$, O145:H$^-$, and O?:H19 in Japan (Kudoh et al., 1994); O26:H11 in the Czech Republic (Bielaszewská et al., 1990), USA (Brown et al., 1998), and Ireland (McMaster et al., 2001); O103:H2 in France (Mariani-Kurkdjian et al., 1993); O104:H21 in the USA (Centers for Disease Control, 1995); O111:H$^-$ in Australia (Cameron et al., 1995), Italy (Caprioli et al., 1994), USA (Banatvala et al., 1996; Centers for Disease Control, 2000), Spain (Blanco et al., 1996), and France (Boudailliez et al., 1997; Mariani-Kurkdjian et al., 1997); O113:H21 in Australia (Paton et al., 1999); and O119 in France (Deschenes et al., 1996). A clone of sorbitol fermenting O157:H7 STEC/VTEC has been isolated from patients with diarrhea and HUS (Gunzer et al., 1992; Karch et al., 1993) and caused an outbreak in Germany (Ammon et al., 1999). Studies in Europe indicate that non-O157 STEC/VTEC strains are increasing in frequency as a cause of hemolytic-uremic syndrome (HUS) and found much more commonly in children with diarrhea (Verweyen et al., 1999; Scheutz et al., 2001; Tozzi et al., 2001).

Among the over 400 STEC/VTEC serotypes, and apart from O157:H$^-$ and O157:H7, those in O groups O26, O103, O111, and O145 are most commonly isolated from humans worldwide. These, along with strains that have caused outbreaks, are clearly recognized as pathogens. Table BXII.γ.205 shows the non-O157 STEC/VTEC serotypes that have been isolated from humans.

E. coli in human extraintestinal infections

Extraintestinal pathogenic E. coli (ExPEC) Extraintestinal pathogenic *E. coli* (ExPEC) are *E. coli* strains that possess currently recognized extraintestinal virulence factors or have been demonstrated to possess enhanced virulence in an appropriate animal model (Russo and Johnson, 2000). ExPEC strains primarily belong to pathogenic clones of a limited number of O:K:H serotypes (Ørskov and Ørskov, 1975), usually with MR fimbriae (P and S fimbriae), siderophores (e.g., aerobactin), host defense-avoidance mechanisms such as capsules (Ørskov and Ørskov, 1977; Ørskov et al., 1982), O antigens, and serum resistance and toxins (often α-hemolysin). ExPEC is common in all age groups and may occur at almost any extraintestinal site. The most common infections include urinary tract infections (UTIs) ranging from uncomplicated to febrile to invasive, pyelonephritis, neonatal, and postneurosurgical meningitis and septicemia. This group is epidemiologically and phylogenetically distinct from commensal and intestinal strains of *E. coli* (Picard et al., 1999). Virulence genes are often located on pathogenicity islands (PAIs), which have the tendency to delete with high frequencies or may undergo duplications and amplifications. They are often associated with tRNA loci, which may represent target sites for the chromosomal integration of these elements (Hacker et al., 1997). Many produce toxins that can lyse erythrocytes of different mammalian species. The best characterized of these is α-hemolysin, which is often produced by strains causing UTIs (J.R. Johnson, 1991) and is believed to play an important role. α-Hemolysin is secreted and can be demonstrated in culture fluid filtrates (Beutin, 1991). Hemolytic colonies can also be identified by the clear zone of hemolysis produced on blood agar plates after 3–4 h of incubation.

Uropathogenic E. coli (UPEC) This somewhat misleading acronym (clones or virulence factors are not syndrome-specific) has been used to refer to the majority of specific clonal groups of uropathogenic *E. coli* isolated from UTIs including pyelonephritis. They are characterized by a number of virulence factors that together play a role in their pathogenesis. First, UPEC is dominated by a limited number of O groups with O groups O1, O2, O4, O6, O7, O18ac, O75, O16, and O15 as the most commonly isolated (Ørskov and Ørskov, 1985). These strains are also represented by a limited number of K antigens: K1, K2, K3, K5, K12, and K13. Common serotypes include O1:K1:H7, O2:K1:H4, O4:K12:H1, O4:K12:H5, O6:K2:H1, O6:K5:H1, O6:K13:H1 (cystitis), O16:K1:H6, and O18ac:K5:H7. Furthermore, the majority of UPECs express P fimbriae of F types F7 through F16 and /or S fimbriae.

Neonatal meningitis E. coli (NMEC) Neonatal meningitis *E. coli* is frequently associated with O groups O7, O18ac, O1, and O6 that have the K1 antigen identical to the capsule of *N. meningitidis* type B (Sarff et al., 1975). O83:K1 strains are also common but apparently only in Europe (Ørskov and Ørskov, 1985). One of the most commonly isolated types is an S fimbriated clone of serotype O18ac:K1:H7.

E. coli in animal infections

As is the case in humans, certain strains of *E. coli* can cause disease in animals. In farm animals, *E. coli* strains are associated with a variety of pathological

TABLE BXII.γ.205. Serotypes of non-O157 STEC/VTEC isolated from humans[a,b,c]

Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype
O1:H⁻	**O8:H21**	**O25:K2:H2**	O52:H23	**O83:H1**	O103:H18	O114:H4	O126:H20	O146:H11	O169:H⁻
O1:H1	O8:H25	O25:H14	O52:H25	**O84:H⁻**	O103:H21	O114:H48	O126:H21	O146:H14	**O171:H⁻**
O1:H2	**O9ab:H⁻**	**O26:H⁻**	O54:H21	O84:H2	O103:H25	**O114:H?**	**O126:H27**	O146:H21	O171:H2
O1:H7	O9:H7	O26:H2	**O55:H⁻**	O84:H20	O103:HNT	**O115:H10**	O127	O146:H28	**O172:H⁻**
O1:H20	O9:H21	O26:H8	**O55:H6**	**O85:H⁻**	**O104:H⁻**	O115:H18	**O128:H⁻**	O148:H28	**O172:H?**
O1:HNT	**O11:H⁻**	**O26:H11**	**O55:H7**	**O85:H10**	**O104:H2**	O116:H⁻	**O128ab:H2**	O150:H⁻	**O173:H2**
O2:H⁻	**O11:H2**	O26:H12	O55:H9	**O85:H23**	O104:H7	O116:H4	**O128:H7**	O150:H8	**O174:H⁻** [d]
O2:H1	O11:H8	O26:H32	**O55:H10**	**O86:H⁻**	O104:H16	O116:H10	O128:H8	O150:H10	**O174:H2**[d]
O2:K1:H2	O11:H49	O26:H46	O55:H19	O86:H10	**O104:H21**	O116:H19	O128:H10	O152:H4	O174:H8[d]
O2:H5	O12:H⁻	O27:H⁻	**O55:H?**	O86:H40	**O105ac:H18**	O117:H⁻	O128:H12	O153:H2	**O174:H21**[d]
O2:H6	**O14:H⁻**	O27:H30	O60:H⁻	O87:H16	O105:H19	**O117:H4**	**O128:H25**	O153:H11	O175:H16[e]
O2:H7	**O15:H⁻**	O28ab:H⁻	O64:H25	O88:H⁻	O105:H20	O117:H7	O128:H31	O153:H12	OX176:H⁻[f]
O2:H11	**O15:H2**	O28:H25	O65:H16	**O88:H25**	O106	O117:K1:H7	**O128:H45**	O153:H21	**OX177:H⁻** [f]
O2:H27	O15:H8	O28:H35	O68:H⁻	O89:H⁻	O107:H27	O117:H8	O129:H⁻	**O153:H25**	OX177:H11[f]
O2:H29	O15:H27	O30:H2	**O69:H⁻**	O90:H⁻	O109:H2	O117:H19	**O130:H11**	O153:H30	OX178:H7[f]
O2:H44	**O16:H⁻**	O30:H21	O69:H11	**O91:H⁻**	O109:H16	O117:H28	O131:H4	O153:H33	OX179:H8[f]
O3:H10	O16:H6	O30:H23	O70:H11	O91:H4	O110:H⁻	O118:H⁻	O132:H⁻	**O154:H⁻**	OX181:H15[f]
O4:H⁻	O16:H21	O37:H41	O71:H⁻	**O91:H10**	O110:H19	O118:H2	O133:H⁻	**O154:H4**	OX181:H49[c]
O4:H5	O17:H18	O38:H21	O73:H34	O91:H14	O110:H28	**O118:H12**	O133:H53	**O154:H19/20**	ONT:H⁻
O4:H10	O17:H41	O38:H26	O74	O91:H15	**O111:H⁻**	**O118:H16**	**O134:H25**	O156:H⁻	**ONT:H2**
O4:H40	**O18:H⁻**	O39:H4	**O75:H⁻**	**O91:H21**	**O111:H2**	**O118:H30**	**O137:H6**	O156:H4	ONT:H8
O5:H⁻	O18:H7	O39:H8	O75:H1	O91:H40	**O111:H7**	O119:H⁻	**O137:H41**	O156:H7	ONT:H18
O5:H16	O18:H12	O39:H28	**O75:H5**	O91:HNT	**O111:H8**	**O119:H5**	O138:H2	O156:H25	ONT:H19
O6:H⁻	O18:H15	O40:H2	O75:H8	O92:H3	O111:H11	**O119:H6**	O141:H⁻	O156:H27	ONT:H21
O6:H1	**O18:H?**	O40:H8	O76:H7	O92:H11	O111:H21	O119:H25	O141:H2	O156:HNT	**ONT:H25**
O6:H2	O20:H⁻	O41:H2	O76:H19	O95:H⁻	O111:H30	**O120:H19**	O141:H8	**O160:H?**	ONT:H41
O6:H4	O20:H7	O41:H26	**O77:H⁻**	O96:H10	O111:H34	**O121:H⁻**	O142	**O161:H⁻**	ONT:H47
O6:H12	**O20:H19**	O44	O77:H4	**O98:H⁻**	O111:H40	O121:H8	O143:H⁻	O162:H4	**ONT:K39:H48**
O6:H28	**O21:H5**	O45:H⁻	O77:H7	O98:H8	O111:H49	O121:H11	O144:H⁻	O163:H⁻	Orough:H⁻
O6:H29	**O21:H8**	O45:H2	O77:H18	**O100:H25**	**O111:H?**	**O121:H19**	**O145:H⁻**	**O163:H19**	Orough:H2
O6:H31	**O21:H?**	O45:H7	O77:H41	O100:H32	**O112ab:H2**	O123:H19	O145:H4	O163:H25	**Orough:H5**
O6:H34	O22:H⁻	O46:H2	O78:H⁻	**O101:H⁻**	O112:H19	O123:H49	**O145:H8**	**O165:H⁻**	**Orough:K1:H6**
O6:H49	**O22:H1**	**O46:H31**	**O79:H7**	O101:H9	O112:H21	O124:H⁻	O145:H16	O165:H10	Orough:K1:H7
O7:H4	**O22:H5**	O46:H38	O79:H14	O102:H6	O113:H2	O125:H⁻	**O145:H25**	**O165:H19**	**Orough:H11**
O7:H8	**O22:H8**	**O48:H21**	O79:H23	**O103:H⁻**	**O113:H4**	O125:H8	O145:H26	O165:H21	**Orough:H16**
O8:H⁻	O22:H16	**O49:H⁻**	O80:H⁻	**O103:H2**	O113:H5	**O125:H?**	**O145:H28**	**O165:H25**	Orough:H18
O8:H2	O22:H40	**O49:H10**	O81:H?	O103:H4	O113:H7	O126:H⁻	O145:H46	O166:H12	Orough:H20
O8:H9	O23:H7	O50:H⁻	O82:H⁻	O103:H6	**O113:H21**	O126:H2	O145:HNT	O166:H15	Orough:H21
O8:H11	O23:H16	**O50:H7**	O82:H5	O103:H7	O113:H32	O126:H8	O146:H⁻	O166:H28	Orough:H28
O8:H14	O23:H21	O51:H49	O82:H8	O103:H11	O113:H53	O126:H11	**L**	O168:H⁻	Orough:H46
O8:H19	O25:H⁻	O52:H19	O83:H⁻						

[a]Data from Scheutz et al. (2001 and unpublished results); Blanco et al. (2001), WHO (1999).

[b]Serotypes in bold represent strains isolated from patients with HUS.

[c]An updated list of STEC, with literature references, can be found at http://www.microbionet.com.au/frames/feature/vtec/brief01.html

[d]Formerly known as OX3.

[e]Formerly known as OX7.

[f]Provisional designation for new O antigens.

conditions, which include colibacillary diarrhea, colibacillary toxemia in pigs, systemic colibacillosis, coliform mastitis, and UTIs. Colibacillary diarrhea is an acute diarrheal disease due to ETEC infection, which occurs primarily in 1–3-d-old calves, lambs, and piglets. A limited number of O groups are represented among these ETEC strains. In England and Wales, the most common O groups of *E. coli* isolates from pigs with diarrhea are O149, O8, O158, O147, and O157 (Wray et al., 1993). Colibacillary toxemia in pigs can take several forms: shock in weaner syndrome, hemorrhagic enteritis, and edema disease. These disease syndromes are also attributable to *E. coli* belonging to a small number of O groups (O8, O45, O138, O139, O141, and O149). Rapid absorption of endotoxin from the bowel is hypothesized to play a role in the pathogenesis of the shock in weaner syndrome and hemorrhagic enteritis, while the toxin Stx2e/VT2e, which is produced by many of the strains having the above specified O groups, has been shown to play a role in the pathogenesis of edema disease (Macleod et al., 1991). Systemic colibacillosis occurs when septicemic strains of *E. coli* pass through the intestinal or respiratory mucosa into the bloodstream of calves, lambs, and poultry. Once they enter the bloodstream, they can cause either a generalized infection or a localized infection, such as meningitis and/or arthritis in calves and lambs or air sacculitis and pericarditis in poultry. *E. coli* strains are also an important cause of mastitis in cows. Endotoxin is believed to play a role in the inflammatory response observed during this disease.

The roles of various adherence mechanisms and toxins in the pathogenesis of the infections were reviewed by Wray and Woodward (1997). Fimbrial antigens and putative colonization factors associated with strains of *E. coli* causing disease in animals cited include F1, F4 (K88), F5 (K99), F41, F6 (987P), F17, F18, CS31A, F165, M326, C1213, F42, F11, curli, type IV pilins, and Nfa. Toxins produced by *E. coli* causing disease in animals include the heat-labile enterotoxins LTI and LTII, heat-stable enterotoxins STa

and STb, cytotoxic necrotizing factors 1 and 2 (CNF1 and CNF2), and Shiga/Verocytotoxins.

Other typing methods For a description of other methods for subdivision of *E. coli*, i.e., phage typing, colicin typing, biotyping, typing by outer membrane protein (OMP) pattern, typing by antibiotic resistance patterns, and typing by direct hemagglutination, see Ørskov and Ørskov (1984a) and Sussman (1985). Phage typing is very useful for certain antigens because antisera are difficult to produce. This is particularly true for K1 (Gross et al., 1977), K3, K5, K7, K12, K13 (Nimmich et al., 1992), and K95 (Nimmich, 1994). A phage-typing scheme for STEC/VTEC O157:H7 established in 1987 (Ahmed et al., 1987) and extended in 1990 (Khakhria et al., 1990) has proven very useful in the epidemiological surveillance of STEC/VTEC O157 (Frost et al., 1993; Saari et al., 2001) infections and is applicable even in low-technology laboratories. In general, phage typing of O157 should be supplemented with one of the molecular typing methods mentioned below. Because of their discriminatory power, speed, use of commercially available reagents and equipment, and amenability to automation and electronic networking, molecular subtyping methods have become very popular for subtyping of *E. coli*, particularly strains involved in causing outbreaks of foodborne disease. Molecular subtyping methods for *E. coli* O157:H7 and other foodborne bacterial pathogens were reviewed by Barrett (1997). Some recently described methods include macrorestriction endonuclease analysis with PFGE (Preston et al., 2000; Zhang et al., 2000; Swaminathan et al., 2001); detection of insertion sequences and characterization of virulence genes by DNA probes or PCR (Thompson et al., 1998; Zhang et al., 2000); detection of amplified fragment length polymorphisms (Iyoda et al., 1999); computer identification by rRNA gene restriction patterns (Machado et al., 1998); and the analysis of randomly amplified polymorphic DNA (Hopkins and Hilton, 2001). In 1996, a molecular subtyping network in the United States, designated PulseNet, for the electronic comparison of DNA fingerprints generated by macrorestriction endonuclease analysis with PFGE was developed to subtype *E. coli* O157:H7. PulseNet has proved an exceptionally valuable tool for detecting outbreaks of *E. coli* O157:H7 infection. The development of standardized laboratory and data analysis protocols and their successful use in providing surveillance for *E. coli* O157:H7 and other foodborne bacterial pathogens was reviewed by Swaminathan et al. (2001).

Enrichment and Isolation Procedures

Many simple agar media can be used for isolation. Media used for selective isolation from feces usually contain substances that partly or completely inhibit growth of bacteria other than *Enterobacteriaceae* (tetrathionate, deoxycholate, bile salts, etc.). The addition of Maranil (dodecylbenzolsulfonate) at a concentration of 0.005% will inhibit swarming of *Proteus* organisms. For details, see Edwards and Ewing (1972) or Kauffmann (1966) or any catalogue from one of the medium-producing companies. At Statens Serum Institut, Copenhagen, a medium developed in the media department of the institute, bromothymol blue (BTB) agar, is used.[1]

Maintenance Procedures

E. coli strains can be kept viable for many years in beef extract agar stabs (tightly closed, e.g., by corks soaked in melted paraffin wax) or on Dorset egg medium. Cultures are initially incubated at 37°C followed by storage in the dark at room temperature (20–22°C). After a few weeks or months, such cultures often contain many mutational forms such as R forms and acapsular forms; consequently, we prefer to store important cultures in beef broth containing 10% glycerol at −80°C. Screw-capped vials are used for easy access.

Procedures for Testing Special Characters

Kilian and Bülow (1976) found that a very high percentage (97%) of *Escherichia coli* and the majority (57%) of *Shigella* strains, exclusively among the *Enterobacteriaceae*, produce β-glucuronidase (GUD). Prolonged incubation of 28 h increases positivity to 99.5% (Rice et al., 1990), which is in accordance with the presence of the *uidA* (GUD) gene in all *E. coli* strains (McDaniels et al., 1996). This test (referred to as the GUD-, PGUA-, MUG-, GUR-, or GLUase-HR test) therefore is very suitable as a screening test for *E. coli*, with the unfortunate exception of most STEC/VTEC O157 strains, which are phenotypically negative. None of the other four species of the genus *Escherichia* are positive for this enzyme (Rice et al., 1991). Both genotypic and phenotypic assays for glutamate decarboxylase (GAD) used by environmental scientists have been described as highly specific for *E. coli*. Unfortunately, these tests have only recently been shown to exhibit the same specificity on a smaller collection of pathogenic isolates of *E. coli* (Grant et al., 2001).

Differentiation of the genus *Escherichia* from other genera

See Table BXII.γ.193 of the family *Enterobacteriaceae* for characteristics that can be used to differentiate this genus from other genera of the family.

Taxonomic Comments

The identification of *Escherichia* strains seldom causes problems; however, many studies have shown that "*Escherichia* is a genus (or species) made up of phenotypically variable strains" (Farmer and Brenner, 1977). DNA–DNA hybridization studies have been an invaluable tool for solving problems in this field. The genus *Shigella* is closely related to *Escherichia*, and only historical reasons make it acceptable that these two genera are not united. Several typical EIEC types have been found that have pathogenic traits that are similar to those of *Shigella*. The Sereny test (Sérèny, 1957), which demonstrates the capacity to cause keratoconjunctivitis in the guinea pig, typical of *Shigella* strains, is also found in these special *Escherichia* strains. Day et al. (1981) described a tissue culture technique that can be used as a substitute for the Sereny test. Typically, such dysentery-associated *E. coli* strains have O antigens that are closely related or identical to *Shigella* O antigens. Brenner et al. (1972a), by DNA reassociation studies, found species-level relatedness between *Shigella* strains and these special *Escherichia* strains, as well as nonpathogenic *E. coli* strains.

1. Bromothymol blue agar (selective for *Enterobacteriaceae*). Combine the following ingredients: peptone (Orthana Ltd., Copenhagen), 10.0 g; NaCl, 5.0 g; yeast extract (Oxoid), 5.0 g; and distilled water, 1000 ml. The pH is adjusted to 8.0, agar powder is added, and the preparation is autoclaved at 120°C for 20 min. The following components are then added aseptically from sterile stock solutions: Maranil solution [Paste A75 (dodecylben-zolsulfonate), Henkel, Germany], 1.0 ml; sodium thiosulfate (50% solution), 2.0 ml; bromothymol blue (Riedel de Haen, Germany; 1.0% solution), 10.0 ml; lactose (33% solution), 27 ml; and glucose (33% solution), 1.2 ml. The pH is adjusted to 7.7–7.8. To obtain optimum results, the amount of glucose must be adjusted for every new batch of yeast extract, peptone, and agar. This medium is very useful for differentiation of lactose-fermenting colonies based on their color.

Not unexpectedly, many strains are phenotypically intermediate between *Escherichia* and *Shigella*, but for obvious reasons a special taxonomic status for such strains is not warranted. In the older literature the name Alkalescens-Dispar is used, but, as stated by Brenner (1978), this group is virtually indistinguishable from *E. coli* strains and is, in fact, a biogroup of *E. coli* that is anaerogenic, lactose-negative (or delayed), and nonmotile.

While most or all characters that classically have been used for definition of the genus *Escherichia* are chromosomally determined, several traits that are not characteristic of *Escherichia* have been found in otherwise typical *Escherichia* strains. Lautrop et al. (1971) and Layne et al. (1971) described H_2S-positive strains of *Escherichia*, and this character was plasmid-determined. It is not known which selective forces account for the simultaneous isolation of H_2S-positive *Escherichia* strains in different parts of the world.

Other "forbidden" phenotypic traits have similarly been described in *Escherichia*, many of which are plasmid-determined. Ørskov et al. (1961) found many urease-producing strains among typical serotypes from piglet diarrhea. Wachsmuth et al. (1979) demonstrated the plasmid-determined nature of a similar urease-positive phenotype in human *E. coli* strains. Citrate-utilizing *E. coli* strains were described by Washington and Timm (1976) and were found to be plasmid determined in similar strains by Sato et al. (1978). Carbon dioxide-dependent cultures can be found (Eykyn and Phillips, 1978). A citrate-positive, malonate-positive biogroup and a biogroup negative in these reactions were described (Burgess et al., 1973).

Further Reading

Abraham, S.N. and S. Jaiswal. 1997. Type-1 fimbriae of *Escherichia coli*. *In* Sussman (Editor), *Escherichia coli*: Mechanisms of Virulence, Cambridge University Press, Cambridge. pp. 169–192.

Cowan, S.T., K.J. Steel, G.I. Barrow and R.K.A. Feltham (Editors). 1995. Cowan and Steel's Manual for the Identification of Medical Bacteria, University of Cambridge, Cambridge.

de Graaf, F.K. and W. Gaastra. 1997. Fimbriae of enterotoxigenic *Escherichia coli*. *In* Sussman (Editor), *Escherichia coli*: Mechanisms of Virulence, Cambridge University Press, Cambridge. pp. 193–211.

Dodson, K.W., F. Jacob-Dubuisson, R.T. Striker and S.J. Hultgren. 1997. Assembly of adhesive virulence-associated pili in Gram-negative bacteria. *In* Sussman (Editor), *Escherichia coli*: Mechanisms of Virulence, Cambridge University Press, Cambridge. pp. 213–236.

Duffy, G., P. Garvey and D.A. McDowell (Editors). 2001. Verocytotoxigenic *E. coli*, Food & Nutrition Press, Inc., Conneticut. pp. 1–457.

Ewing, W.H. 1986. The Genus *Escherichia*. *In* Edwards and Ewing (Editors), Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th Ed., Elsevier Science Publishing Co., New York. pp. 93–122.

Gyles, C.L. (Editor). 1994. *Escherichia coli* in Domestic Animals and Humans, CAB International, Wallingford.

Jann, K. and B. Jann (Editors). 1990. Bacterial Adhesins. Current Topics in Microbiology and Immunology, Springer-Verlag, Berlin. 151 pp.

Kaper, J.B. and A.D. O'Brien (Editors). 1998. *Escherichia coli* O157:H7 and other Shiga Toxin-Producing Strains, ASM Press, Washington, D.C. 465 pp.

Klemm, P. (Editor). 1994. Fimbriae: Adhesins, Genetics, Biogenesis, and Vaccines, CRC Press, Boca Raton.

Low, D., B. Braaten and M. van der Woude. 1996. Fimbriae. *In* Neidhardt, Curtiss, Ingraham, Lin, Low, Magasanik, Reznikoff, Riley, Schaechter and Umbarger (Editors), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd Ed., ASM Press, Washington, D.C. pp. 146–157.

Nataro, J.P. and J.B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. *11*: 142–201.

Neidhardt, F.C., R. Curtiss, III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter and H.E. Umbarger (Editors), 1996. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd Ed., ASM Press, Washington, D.C.

Sussman, M. (Editor). 1997. *Escherichia coli*: Mechanisms of Virulence, Press syndicate of the University of Cambridge, Cambridge. 639 pp.

Differentiation of the species of the genus *Escherichia*

Characteristics useful in distinguishing the five species of *Escherichia* are given in Table BXII.γ.193 of the family *Enterobacteriaceae* and in Table BXII.γ.197 of the genus *Escherichia*.

List of species of the genus Escherichia

1. **Escherichia coli** (Migula 1895) Castellani and Chalmers 1919, 941[AL] (*Bacillus coli* Migula 1895, 27.)
co' li. Gr. n. *colon* large intestine, colon; M.L. gen. n. *coli* of the colon.

The characteristics are as described for the genus and as listed in Table BXII.γ.193 of the family *Enterobacteriaceae*. Occurs naturally in the lower part of the intestine of warm-blooded animals, and as intestinal (some foodborne) and extraintestinal pathogens of humans and animals.

The mol% G + C of the DNA is: 48.5–52.1 (T_m).

Type strain: ATCC 11775, CCM 5172, CIP 54.8, DSM 30083, IAM 12119, NCDO 1989, NCTC 9001. Serotype O1:K1(L1):H7.

GenBank accession number (16S rRNA): X80725.

Additional Remarks: Other sequences are listed in Table BXII.γ.206.

2. **Escherichia blattae** Burgess, McDermott and Whiting 1973, 4[AL]
blat' tae. L. fem. n. *blatta* cockroach; L. gen. n. *blattae* of the cockroach.

The characteristics are as described for the genus and as listed in Table BXII.γ.193 of the family *Enterobacteriaceae*. *E. blattae* was isolated from the hindgut of healthy cockroaches, *Blatta orientalis*, in England (Burgess et al., 1973) and on Easter Island (Nogrady and Aubert, personal com-

TABLE BXII.γ.206. *rrn* operon sequences of *Escherichia* strains

Source and strain[a, b]	EMBL[c]	Method[d]
Escherichia coli:		
	J01859	rRNA
	J01695	*rrn*B
	V00348	*rrn*B
(PK3)	X80731	PCR
(MC4100)	X80732	PCR
CIP (ATCC 11775^T)	X80725	PCR
ATCC 25922	X80724	PCR
(K-12)	M87049	*rrn*A
(K-12)	U00006	*rrn*B
(K-12)	L10328	*rrn*C
(K-12)	U18997	*rrn*D
(K-12)	U00006	*rrn*E
	M29364	*rrn*G
(K–12)	D15061	*rrn*H
BioM	X80733	PCR
(PK3)	X80721	*rrn*A
(PK3)	X80722	*rrn*B
(PK3)	X80723	*rrn*C
(PK3)	X80727	*rrn*D
(PK3)	X80728	*rrn*E
(PK3)	X80729	*rrn*G
(PK3)	X80730	*rrn*H
Escherichia fergusonii:		
ATCC 35469	AF530475	NA[e]
Escherichia hermannii:		
BioM	X80675	rRNA
Escherichia vulneris:		
CIP (ATCC 33821^T)	X80734	PCR

[a]Some strain numbers have been lost; sequences were most probably obtained using *E. coli* K-12.

[b]Bacterial collection from which each strain is deposited: ATCC (American Type Culture Collection); BioM (BioMérieux, Marcy l'Étoile, France), CIP (Collection de l'Institut Pasteur).

[c]Accession numbers under which sequence is available.

[d]Method by which each sequence has been obtained: rRNA (total rRNA sequenced using reverse transcriptase), PCR (total PCR products sequenced using T7-DNA polymerase); *rrn*X (sequence of a single operon, X).

[e]Not available.

munication). It appears as two biotypes, one of which is citrate and malonate positive, the other negative, and it is the only species within *Escherichia* that is gluconate positive (Burgess et al., 1973). *E. blattae* has not been associated with disease either in humans or in cockroaches.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 29907, CDC 9005-74, DSM 4481, NCTC 12127.

GenBank accession number (16S rRNA): X87025.

3. **Escherichia fergusonii** Farmer, Fanning, Davis, O'Hara, Riddle, Hickman-Brenner, Asbury, Lowery and Brenner 1985c, 223^{VP} (Effective publication: Farmer, Fanning, Davis, O'Hara, Riddle, Hickman-Brenner, Asbury, Lowery and Brenner 1985b, 77.)*
fer.gu.so' ni.i. M.L. masc. (substantive) *fergusonii* coined to honor the American microbiologist William W. Ferguson, who made many contributions to enteric bacteriology and was one of the first to show the role of certain strains of *E. coli* in infantile diarrhea (Farmer et al., 1985b).

The characteristics are as described for the genus and as listed in Table BXII.γ.193 of the family *Enterobacteriaceae*. Has been isolated from human clinical specimens (stool, urine, blood, and an abdominal wound), the feces of captive raptors belonging to the order *Falconiformes* or *Strigiformes* (Bangert et al., 1988), and from unspecified sites for other warm-blooded animals.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 35469, CDC 0568-73.

Additional Remarks: Other sequences are listed in Table BXII.γ.206.

4. **Escherichia hermannii** Brenner, Davis, Steigerwalt, Riddle, McWhorter, Allen, Farmer, Saitoh and Fanning 1983a, 438^{VP} (Effective publication: Brenner, Davis, Steigerwalt, Riddle, McWhorter, Allen, Farmer, Saitoh and Fanning 1982a, 705.)*
her.man' ni.i. M.L. *hermannii* of Hermann, named in honor of George J. Hermann, former chief of the Enteric Section at the CDC, for his many contributions to enteric bacteriology, and Lloyd G. Herman, formerly of the Environmental Services Branch, National Institutes of Health, Bethesda, MD, for his contributions to the study of yellow-pigmented bacteria (Brenner et al., 1982a).

The characteristics are as described for the genus and as listed in Table BXII.γ.193 of the family *Enterobacteriaceae*. Those that together distinguish it from most other members of this family include growth in the presence of KCN, fermentation of cellobiose, and production of yellow pigment. Has been isolated from human clinical specimens (wounds, sputum, lung, stool, blood, and spinal fluid) and recently from the sludge of an industrial wastewater treatment plant (Kiernicka et al., 1999). The sludge isolate shows promise for bioremediation; it grows in and degrades high concentrations of chlorobenzene.

The mol% G + C of the DNA is: 53–58 (T_m).

Type strain: ATCC 33650, CDC 980-72, DSM 4560.

Additional Remarks: Other sequences are listed in Table BXII.γ.206.

5. **Escherichia vulneris** Brenner, McWhorter, Leete Knutson and Steigerwalt 1983d, 438^{VP} (Effective publication: Brenner, McWhorter, Leete Knutson and Steigerwalt 1982b, 1137.)*
vul.ner' is. L. n. *vulnus* a wound; L. gen. n. *vulneris* of a wound; *Escherichia vulneris* the *Escherichia* of a wound.

The characteristics are as described for the genus and as listed in Table BXII.γ.193 of the family *Enterobacteriaceae* (Brenner et al., 1982b). Has been isolated from human clinical specimens, primarily wounds, the majority of which occurred on the arms or legs, but also blood, throat, sputum, vagina, urine, and stool, and other warm-blooded animals. The type species was isolated from the intestine of a cowbird in Michigan, USA.

The mol% G + C of the DNA is: 58.5–58.7 (T_m).

Type strain: ATCC 33821, CDC 875-72, DSM 4564, NIH 580.

GenBank accession number (16S rRNA): X80734.

Additional Remarks: Other sequences are listed in Table BXII.γ.206.

**Editorial Note:* This species was formerly known as Enteric Group 10.

**Editorial Note:* This species was formerly known as Enteric Group 11.

**Editorial Note:* This species was formerly known as Enteric Group 1.

Genus II. **Alterococcus** *Shieh and Jean 1999, 341VP (Effective publication: Shieh and Jean 1998, 644)*

THE EDITORIAL BOARD

Al.te.ro.coc′cus. L. *alter* another; Gr. n. *coccus* a grain or berry; M.L. masc. n. *Alterococcus* another coccus.

Spherical cells 0.8–0.9 μm in diameter, occurring singly or in pairs. **Motile by means of a single flagellum**. Gram negative. **Facultatively anaerobic**, capable of aerobic and anaerobic fermentation. Chemoheterotrophic. **Oxidase and catalase positive**. Optimum temperature for growth, 45°C. No growth occurs at 30°C or 60°C. **Halophilic**; growth occurs in the presence of 1–3% NaCl (optimum, 2.0%), but not 0% or 5% NaCl. The major cellular fatty acid is *anteiso*-15-carbon acid ($C_{15:0\ anteiso}$). Cause agar liquefaction. Habitat: coastal hot springs.

The mol% G + C of the DNA is: 65.5–67.0.

Type species: **Alterococcus agarolyticus** Shieh and Jean 1999, 341 (Effective publication: Shieh and Jean 1998, 644)

FURTHER DESCRIPTIVE INFORMATION

Butyrate and propionate are formed during both aerobic and anaerobic growth in PY semisolid medium[1]. Butyrate with propionate and/or formate is produced during both aerobic and anaerobic growth in PYG broth[2].

ENRICHMENT AND ISOLATION PROCEDURES

Water samples are decimally diluted with sterile NaCl-MOPSO buffer[3] and spread on PY agar. The plates are incubated aerobically at 50°C in the dark for 3–7 d. Colonies that cause agar liquefaction on the incubated plates are purified by successive streaking.

MAINTENANCE PROCEDURES

Early stationary-phase cultures grown in PY broth are inoculated into sterile 60% seawater at a ratio of 0.25 ml in 5 ml (1:20) and stored for two or three months at 45°C.

DIFFERENTIATION OF THE GENUS *ALTEROCOCCUS* FROM OTHER GENERA

The genus is the only one known to contain Gram-negative, halophilic, thermophilic bacteria that degrade agar and grow both aerobically and anaerobically. The only other Gram-negative genera that contain heterotrophic, halophilic, thermophilic bacteria are *Rhodothermus*, *Thermotoga*, *Thermosipho*, and *Spirochaeta*. *Rhodothermus* contains obligately aerobic rods, whereas *Alterococcus* contains cocci. The genera *Thermotoga* and *Thermosipho* contain strictly anaerobic rods, whereas *Alterococcus* is facultatively anaerobic. *Spirochaeta* contains facultatively anaerobic species, but the cells are spiral, not coccoid.

TAXONOMIC COMMENTS

16S rDNA-based phylogenetic analysis of the type strain of *Alterococcus agarolyticus* has indicated that the species is most closely related to members of the family *Enterobacteriaceae*. In this edition of the *Manual*, the genus is placed in this family, which belongs to the order *Enterobacteriales* in the class *Gammaproteobacteria*.

List of species of the genus Alterococcus

1. **Alterococcus agarolyticus** Shieh and Jean 1999, 341VP (Effective publication: Shieh and Jean 1998, 644.)
   a.gar.o.ly′ti.cus. Malayan n. *agar* agar, a complex gelling polysaccharide from marine red algae; Gr. adj. *lyticum* dissolving; M.L. adj. *agarolyticus* agar-dissolving.

   The characteristics are as described for the genus and as listed in Table BXII.γ.207. Young colonies on agar media are white, circular, and opaque. Glucose, cellobiose, galactose, lactose, sucrose, trehalose, and xylose are fermented. Sensitive to ampicillin, chloramphenicol, erythromycin, penicillin G, and tetracycline.

   Isolated from two hot springs in the intertidal zone of Lutao Island, Taiwan.

   The mol% G + C of the DNA is: 65.8.

   Type strain: ADT3, CCRC 19135.

   GenBank accession number (16S rRNA): AF075271.

TABLE BXII.γ.207. Phenotypic characteristics of *Alterococcus agarolyticus*[a,b]

Characteristic	Reaction
Fermentation of:	
Adonitol, D-arabinose, dulcitol, *myo*-inositol, mannitol, melibiose, sorbitol	−
Cellobiose, galactose, glucose, lactose, sucrose, trehalose, xylose	+
Mannose	W
Motility	+
Swarming	−
Luminescence	−
Agarase activity	+
Hydrolysis of starch	W
Hydrolysis of casein, DNA, gelatin, fats	−
Catalase, oxidase	+
Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase	−
Growth at:	
30°C	−
38°C	W
40–56°C	+
58°C	W
60°C	−
Growth in presence of NaCl levels of:	
0%	−
0.50%	W
1.0–3.5%	+
4.00%	W
5.00%	−

[a]Symbols: +, positive; −, negative; W, weakly positive.

[b]Data from Shieh and Jean (1998).

1. PY broth is composed of (g/l deionized water): Bacto Peptone (Difco), 4.0; Bacto yeast extract (Difco), 2.0; NaCl, 20.0; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.01; and 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO; Sigma), 4.5. This broth medium is adjusted to pH 7.0. Bacto agar (Difco) is added to this medium at 5 and 15 g/l for the preparation of PY semisolid and plating media, respectively.

2. PYG medium is composed of two parts. Part 1 consists of the following ingredients dissolved in 900 ml of distilled water: Bacto Peptone (Difco), 4.0 g; Bacto yeast extract (Difco), 2.0 g; NaCl, 20.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2$, 0.01g; and Tris buffer (Sigma), 6.0 g. The pH is adjusted to 7.8. Part 2 consists of glucose (5.0 g) dissolved in 100 ml of distilled water. Parts 1 and 2 are autoclaved separately and combined aseptically after cooling to room temperature.

3. NaCl-MOPSO buffer consists of (g/l deionized water): NaCl, 25.0 and MOPSO (*N*-morpholino)-2-hydroxypropanesulfonic acid), 0.45; pH 7.0.

Genus III. **Arsenophonus** *Gherna, Werren, Weisburg, Cote, Woese, Mandelco and Brenner 1991, 564*[VP]

JOHN H. WERREN

Ar.se.no.pho'nus. Gr. n. *arsen* a male; Gr. suff. *phonus* slayer; N.L. masc. n. *Arsenophonus* male-killer.

Cells are Gram-negative, nonmotile, nonsporeforming, nonflagellated, long to highly filamentous rods, which divide by septation. **The bacteria infect insect tissues.** The type species can be cultivated on cell-free media. Colonies are mucoid, gray-white, round, and convex with entire edges. Enzymatically digested proteins best serve as nitrogen sources. Does not utilize KNO_3, $(NH_4)_2SO_4$, complete defined amino acid mixtures, or acid-hydrolyzed peptones as nitrogen sources. Sugars sucrose, fructose, and D-glucose are utilized as primary carbon sources. Weak growth occurs with maltose, trehalose, cellobiose, and D-xylose. Acid is produced with D-glucose, fructose, and sucrose. Growth is negative with L-arabinose, glycerol, dulcitol, lactose, D-mannitol, raffinose, and *i*-inositol. Positive for catalase and gelatin liquefaction; negative for Voges–Proskauer, methyl red, nitrate reduction, indole, oxidase, hydrogen sulfide, O-nitrophenyl-β-D-galactopyranoside, lysine and ornithine decarboxylase, urease, and arginine dehydrolase. Cells grow at pH 6.2–8.7 (optimum pH range 7.4–8.0), and a temperature range of 15–35°C (30°C optimum).

There is currently only one validly named species in the genus (and an additional *Candidatus* species). However, **the genus may be widespread in insects**, based upon recent evidence. Therefore, the genus description may well change as additional information becomes available.

The mol% G + C of the DNA is: 39.5.

Type species: **Arsenophonus nasoniae** Gherna Werren, Weisburg, Cote, Woese, Mandelco and Brenner 1991, 564.

FURTHER DESCRIPTIVE INFORMATION

General biology The genus *Arsenophonus* was originally described from a single bacterial species that is the causative agent of the "son-killer" trait in the parasitic wasp *Nasonia vitripennis* (insect order *Hymenoptera*). Subsequently, a second species has been proposed for the genus (*Candidatus* Arsenophonus triatominarum), isolated from a reduviid bug (insect order *Heteroptera*). Despite being found in divergent insects, the two are similar in morphology, patterns of infection of host insect tissues, and 16S rDNA sequence (97.8% similarity). Biochemical characterization of "*Candidatus* Arsenophonus triatominarum" has not yet

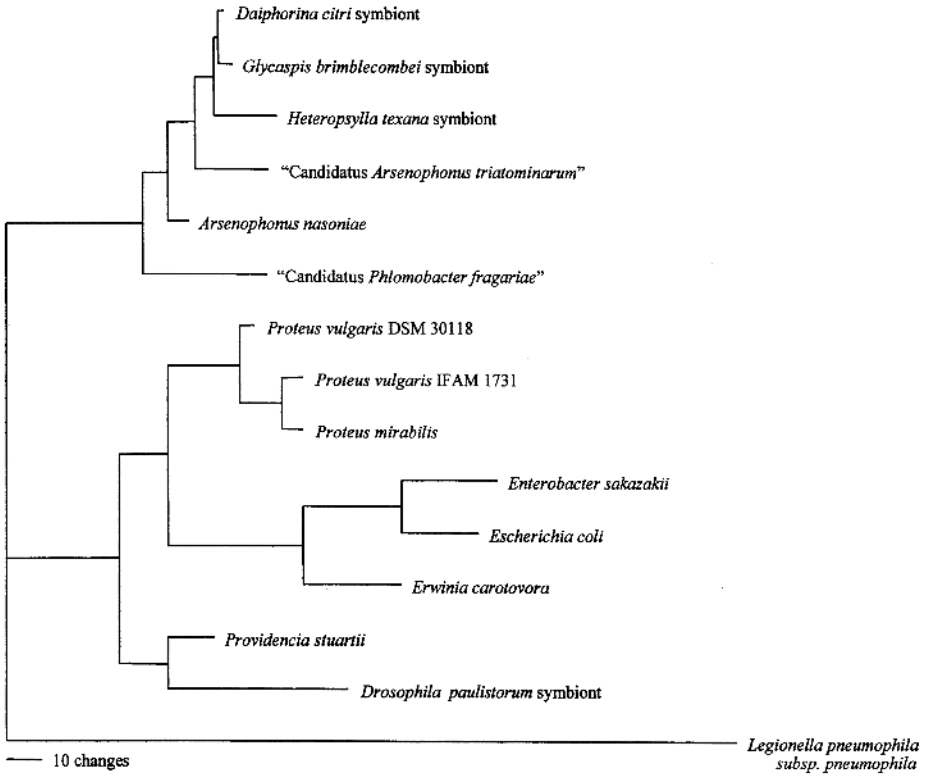


FIGURE BXII.γ.193. A phylogenetic tree based on 16S rDNA sequences is presented based on a parsimony analysis using a heuristic search and stepwise addition (D.L. Swofford, 1996, PAUP 4.0 Sinauer, Sunderland, MA) with *Legionella pneumophila* defined as the outgroup. Partial 16S rDNA nucleotide sequences (1476 sites) of *Arsenophonus nasoniae, Candidatus* A. triatominarum, other endosymbiotic and free-living bacteria were analyzed. Names of the insect host species are shown for the psyllid secondary symbionts and the unidentified bacterium from *D. paulistorum*. In all other cases the bacterial genus and species are shown. Numbers above nodes are bootstrap percentages from 1000 replicate searches. The sequence data for *Diaphorina citri* secondary symbiont was provided by T. Fukatsu (Subandiyah et al., 2000). Other sequences were obtained from the NCBI database (accession numbers AF263561, AF263562, U91786, M90801, U91515, X07652, AJ233425, X07652, AF008582, AB004746, AB035924, AJ233411, AF008581, U20273, and X73402).

been performed; nor are its effects on the host insect known. *A. nasoniae* can be grown on cell-free media; "*Candidatus* A. triatominarum" has not yet been grown in cell-free culture, but can be maintained on insect tissue culture.

Phylogenetic analysis using 16S rDNA sequences (see below) indicates that closely related bacteria are secondary symbionts in several species of psyllid insects (insect order *Homoptera*), although the taxonomic status of these bacteria has not yet been resolved. Taken together, the results suggest that *Arsenophonus* (and related bacteria) may have a wide distribution as infectious agents of insects. Another closely related bacterium, *Candidatus* Phlomobacter fragariae, causes disease in strawberry (Zreik et al., 1998).

Phylogenetic analyses Based on 16S rDNA phylogeny *Arsenophonus* is a lineage in the *Gammaproteobacteria* (Gherna et al., 1991; Hypsa and Dale, 1997), within the family *Enterobacteriaceae*. The phylogenetic analysis places *A. nasoniae* and the *Candidatus* A. triatominarum as close relatives (97.8% similarity) (Fig. BXII.γ.193). Other closely related organisms (Fig. BXII.γ.193) are secondary symbionts found in psyllid insects (98.6–97.6% similarity). Although they are closely related, the specific phylogenetic relationships between *A. nasoniae*, *Candidatus* A. triatominarum, and the psyllid secondary symbionts are not clearly resolved. These may eventually be placed in the genus *Arsenophonus* when further characterization is completed. *A. nasoniae*, *Candidatus* A. triatominarum, and the three psyllid secondary endosymbionts form a monophyletic clade with *Candidatus* Phlomobacter fragariae (97.0% similarity to *A. nasoniae*). This monophyletic group is strongly supported by various phylogenetic analyses. *Candidatus* Phlomobacter fragariae is found within the phloem of strawberry and is associated with strawberry marginal chlorosis disease (Zreik et al., 1998). Plant phloem associated bacteria can be vectored by phloem feeding insects such as psyllids and aphids, which may suggest a biological basis for its phylogenetic similarity to psyllid secondary endosymbionts. The apparent monophyly of this bacterium with *Arsenophonus* and the psyllid secondary endosymbionts (Subandiyah et al., 2000; Thao et al., 2000b) suggests that separate generic status for this bacterium may not be warranted. However, additional sequences and biological characterization are needed to resolve the issue. The closest known bacteria outside of the "*Arsenophonus* group" include *Proteus vulgaris*, *Proteus mirabilis*, and *Providencia stuartii* (94.2–94.3% similarity).

List of species of the genus Arsenophonus

1. **Arsenophonus nasoniae** Gherna, Werren, Weisburg, Cote, Woese, Mandelco and Brenner 1991, 564[VP]
na.so′ni.ae. N.L. n. *Nasonia* a genus of parasitoid wasps; *nasoniae* of the genus *Nasonia*.

   The cells are nonmotile nonsporeforming, long rods to highly filamentous (0.40–0.57 × 6.9–10.0 μm) in culture. Filamentous cells can also be found in insect tissues. Biochemical features are presented in the genus description. *A. nasoniae* is the causative agent of the "son-killer" trait in the parasitic wasp *N. vitripennis*. *Nasonia* wasps parasitize the pupae of various dipterans (flies). They sting the host pupa and lay their eggs underneath the puparial wall. The insects hatch and feed upon the host fly to complete development. In female wasps infected with the bacterium, male eggs fail to hatch, whereas female eggs develop normally (Werren et al., 1986). Genetic experiments indicate that the bacterium acts by blocking the development of unfertilized eggs, which normally develop into males in this haplodiploid species. The son-killer bacterium is found in *N. vitripennis* populations throughout North America at frequencies around 10%, and has also been detected in the sibling species *N. longicornis* (Balas et al., 1996).

   The bacterium is introduced into the dipteran host pupa during stinging by the infected female, where it replicates and is ingested by the feeding wasp larvae (Huger et al., 1985). As a result, the bacteria can be transmitted maternally from an infected female to her developing larvae, and horizontally when more than one female parasitizes the same host. The bacteria initially invade the midgut epithelium and subsequently spread to various tissues, including brain, fat body, muscles, eyes, and hemocytes. Massive infections of tissues can be observed in adults without major negative effects on insect viability. In insect tissues, the bacteria are pleomorphic, with rods being the most common morphology. Both male and female larvae can become infected by host feeding during larval development. Female reproductive tracts are often infected with the bacteria, whereas infections are not observed in male reproductive tissues. In adult wasps, drop-like bacterial masses can be discharged from the midgut epithelium into the gut lumen, suggesting a possible additional fecal route for infectious transmission. The mechanism by which *A. nasoniae* causes lethality of male eggs is unknown. Experiments indicate that unfertilized eggs, which normally develop into males in the haplodiploid insect host, fail to develop when the female is infected. Levels of male embryo mortality can vary in different infected females (Balas et al., 1996).

   A. nasoniae can be isolated from infected insects by surface sterilization of the insect followed by maceration of the insect onto GC media with Kellogg's supplement, and serial streaking of the macerated material. Characteristic gray-white mucoid colonies will develop in 2–4 d at 26°C. Bacteria can also be isolated by antiseptic removal of hemolymph from fly pupae parasitized by infected *Nasonia* females, and inoculation of the hemolymph onto GC media with Kellogg's supplement.

   The type strain grows poorly or does not grow on many standard media. It can be grown on brain–heart infusion broth (Difco no. 0037) or solid media. However, improved growth was found on a GC media base (Difco no. 0289) supplemented with Kellogg's additive (Kellogg et al., 1963). Even on this medium, bacterial growth is slow relative to many culturable bacteria, and visible colonies may take 2–3 d at 26°C. Growth is best on solid media. Broth cultures show elevated frequencies of filamentous bacteria relative to agar base solid media. The type strain was isolated from an *N. vitripennis* son-killer strain collected near Salt Lake City, Utah.

   The mol% G + C of the DNA is: 39.5 (T_m).
   Type strain: SKI4, ATCC 49151.
   GenBank accession number (16S rRNA): M90801.

2. *Candidatus* Arsenophonus triatominarum Hypsa and Dale 1997, 1143.
tri′a.to′min.a.rum. N.L. n. *Triatominae* subfamily of reduviid bugs; *triatominarum* of the *Triatominae*.

   Cells are nonmotile, nonsporeforming, nonflagellated highly filamentous rods (1–1.5 × >15 μm), and divide by septation. Bacteria grow within the cytoplasm of cells of the reduviid bug *Triatoma infestans*. Phylogenetic analysis of 16S ribosomal gene sequence indicates a close phylogenetic re-

lationship to *A. nasoniae* (97.8% similarity), as do general morphological characteristics and the pattern of infection within tissues of their respective hosts. The bacterium cannot be grown on cell-free minimal medium, but can be maintained in *A. albopictus* cell culture. Biochemical characteristics of the bacterium have not yet been determined.

"*Candidatus* A. triatominarum" was isolated from the hemolymph of *Triatoma infestans*, a reduviid bug also known to be a vector of *Trypanosoma cruzi*, the causative agent of Chagas disease in humans. Within infected *T. infestans*, heavy infections can be found in neural ganglia, visceral muscles, salivary glands, nephrocytes, testes, ovaries, and dorsal vessels. In contrast to *A. nasoniae*, the bacterium could not be grown of a variety of tested cell-free media, including media upon which *A. nasoniae* can be cultured (Hypsa and Dale, 1997). Phenotypic effects of the bacterium on *T. infestans* are unknown. Strain TI1 was isolated from hemolymph of *T. infestans* individuals from a laboratory colony, and cultured in *Aedes albopictus* cell line C6/36.

The mol% G + C of the DNA is: unknown.

Deposited strain: TI1.

GenBank accession number (16S rRNA): U91786.

Genus IV. **Brenneria** *Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1^VP (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 394.)*

LYSIANE HAUBEN AND JEAN SWINGS

Bren.ne' ri.a. M.L. fem. n. *Brenneria* named after Don J. Brenner.

Cells are 0.5–1.0 × 1.3–3.0 μm; have rounded ends; occur singly or rarely in pairs. Gram negative. Motile by peritrichous flagella. Facultatively anaerobic, but anaerobic growth by some species is weak. Optimum temperature, 27–30°C; maximum temperature for growth is 40°C. Oxidase negative. Catalase positive. Acid is produced from fructose, D-galactose, D-glucose, D-mannose, salicin, and sucrose but not from adonitol or dulcitol. Do not possess arginine decarboxylases, arginine dihydrolase, lysine decarboxylases, ornithine decarboxylases, or starch hydrolase.

The species of the genus *Brenneria* comprise a distinct phylogenetic group, as determined by 16S rRNA gene sequence comparisons, and have 12 characteristic signature nucleotides (Table BXII.γ.223 of the genus *Erwinia*).

Brenneria species cause diseases on trees (e.g., deciduous trees and walnut trees), which include blights, cankers, wilts, necrosis, and rots. Ingress by the pathogen generally occurs through natural openings and wounds. More details on disease symptoms are described for each species.

The mol% G + C of the DNA is: 50.1–56.1 (T_m, Bd).

Type species: **Brenneria salicis** (Day 1924) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 394) (*Erwinia salicis* (Day 1924) Chester 1939, 406; *"Bacterium salicis"* Day 1924, 14.)

FURTHER DESCRIPTIVE INFORMATION

Metabolic features are the same as for the genus *Erwinia*.

Pectate lyases are produced by strains of *B. rubrifaciens* (Gardner and Kado, 1976).

Fermentation end products from D-glucose are CO_2 and different combinations of succinate, lactate, formate, and acetate; some form 2,3-butanediol and some ethanol (White and Starr, 1971). Starch is not hydrolyzed beyond dextrins.

16S rDNA sequence analyses of the species of the genus *Brenneria* by Kwon et al. (1997) and Hauben et al. (1998a) are in good agreement except for the type strain of *B. salicis*, and place the genus *Brenneria* within the *Enterobacteriaceae*, closely related to the genera *Pectobacterium*, *Erwinia*, *Pantoea*, and *Enterobacter* (Fig. BXII.γ.201 and Table BXII.γ.223 of the genus *Erwinia*). The gene sequences of the type strains of *B. salicis* sequenced by Kwon et al. (1997) (ATCC 15712) and Hauben et al. (1998a) (LMG 2698) differ in 73 nucleotides. A genomic fingerprinting study by AFLP of 77 *B. salicis* strains revealed the profile of strain LMG 2698 to be highly similar (>80% similarity) to the ones of other authentic *B. salicis* strains, indicating that strain LMG 2698 is an authentic *B. salicis* (Hauben et al., 1998b).

Virulent or temperate phages have been isolated, characterized, and reported to be active against strains of *B. nigrifluens* and *B. rubrifaciens* (Zeitoun and Wilson, 1969).

Antisera prepared against live or heat-killed cells, nonpurified or purified immunogens, have been used for the differentiation or identification of all *Brenneria* species (De Kam, 1976; Schaad, 1979).

ENRICHMENT AND ISOLATION PROCEDURES

The isolation procedure is the same as for the genus *Erwinia*. The affected plant material is washed in tap water, then in sterile water, and dried by paper toweling. Surface sterilization (3 min in 1:10 dilution of 5.25% active sodium hypochlorite) is sometimes detrimental for isolation. Affected tissue is removed from a young lesion or the edge of older necrotic areas by a sterile scalpel; the tissue is crushed in sterile water, saline, or buffer solution, and is streaked onto a solid medium.

The isolation of some *Brenneria* species can be facilitated by use of selective-differential media, but such media are usually not necessary. *B. nigrifluens*, *B. quercina*, and *B. rubrifaciens* will grow on MS medium (Miller and Schroth, 1972) and produce characteristic colonies. A soluble pink pigment is produced by *B. rubrifaciens*.

MAINTENANCE PROCEDURES

Stock cultures of *Brenneria* species should be grown on standard media of choice at 25–30°C until good growth occurs. The cultures can be maintained for short-term storage in a refrigerator (4–5°C).

Long-term preservation is the same as for the genus *Erwinia*. The bacteria can be successfully stored as lyophilized cultures, usually suspended in a filter-sterilized mixture of 200 ml of horse serum (Oxoid SR035C) to which 1675 mg of nutrient broth (Oxoid) and 20 g of glucose in 67 ml of distilled water is added. Strains have also been stored in liquid nitrogen and in glycerol at −80°C (broth + 15% glycerol).

DIFFERENTIATION OF THE GENUS *BRENNERIA* FROM OTHER GENERA

The differential characteristics of the species of *Brenneria* are given in Tables BXII.γ.208 and BXII.γ.209.

TABLE BXII.γ.208. Diagnostic reactions for *Brenneria* species[a]

Characteristic	1. *B. salicis*	2. *B. alni*	3. *B. nigrifluens*	4. *B. paradisiaca*	5. *B. quercina*	6. *B. rubrifaciens*
Indole	−	−	−	+	−	−
Methyl red	nd	+	+	nd	−	nd
Growth factors required	nd	−	−	nd	+	nd
Endoglucanase activity	nd	+	−	nd	+	nd
Esculin hydrolase	nd	−	+	nd	−	nd
β-Galactosidase	+	−	v	+	v	+
Pectinase	nd	−	−	+	−	nd
Urease	−	+	v	−	−	−
Acid production from:						
Arabinose	−	+	+	+	−	+
Esculin	−	nd	+	nd	−	−
Maltose	nd	+	−	−	−	+
Melibiose	−	−	+	+	−	nd
Raffinose	+	−	+	+	−	−
Sorbitol	nd	−	+	−	v	−
Trehalose	nd	+	+	nd	−	nd
Xylose	−	+	+	+	−	−
Utilization of carbon sources:						
L-Arabinose	nd	nd	+	+	−	+
Citrate	+	−	v	+	+	+
Galacturonic acid	−	nd	−	+	−	−
Maltose	nd	nd	−	nd	+	−
Raffinose	+	nd	+	nd	−	−
L-Rhamnose	nd	nd	nd	+	nd	−
Sorbitol	−	nd	+	nd	−	−
Trehalose	nd	nd	+	+	−	−
Xylose	−	nd	−	+	−	−
Utilization of nitrogen sources:						
Acetamide	nd	nd	−	nd	−	+
Anthranilic acid	+	nd	−	nd	−	nd
Glycocyamine	nd	nd	+	nd	−	+
L-Serine	+	nd	+	+	−	+
Threonine	+	nd	−	+	+	+
Tryptamine	−	nd	−	+	−	−
Xanthin	+	nd	+	−	nd	+
Sensitivity toward:						
Streptomycin	nd	nd	−	+	nd	nd

[a]For symbols see standard definitions; nd, not determined.

Taxonomic Comments

The species of the genus *Brenneria* were formerly classified in the genus *Erwinia*. We refer to the taxonomic comments of the genus *Erwinia* for a discussion of this issue.

Characteristics that differentiate *Brenneria* from the genera *Pectobacterium*, *Pantoea*, and *Erwinia* are given in Table BXII.γ.223 of the *Erwinia* chapter. As it is very difficult to differentiate them phenotypically, genomic methods are recommended for differentiation.

List of species of the genus Brenneria

1. **Brenneria salicis** (Day 1924) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 394) (*Erwinia salicis* (Day 1924) Chester 1939, 406; *"Bacterium salicis"* Day 1924, 14.)
sa' li.cis. L. n. *salix* the willow; L. gen. n. *salicis* of the willow.

   The characteristics are as given for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

   Grows poorly on nutrient agar but moderately well on YDC agar (0.5% yeast extract, 1% dextrose, 3% $CaCO_3$) or on glucose nutrient agar. Colonies on 0.5% starch potato agar (pH 6.5) are yellowish in 2–3 d. A bright yellow pigment is produced on autoclaved potato tissue. Craters form around colonies on the pectate gel of Paton (1959).

   A PCR based identification and detection method was developed with primers Es1a (5′-GCGGCGGACGGGTGAGTAAA-3′) and Es4b (5′-CTAGCCTGTCAGTTTTGAATGCT-3′), annealing at 64°C, derived from the 16S rDNA sequence of *B. salicis* (Hauben et al., 1998b).

   Antisera have been used for the identification of *B. salicis* (De Kam, 1976; 1986) and soluble antigens were detected in the leaves of *Salix alba* (De Kam, 1982).

   Causes a vascular wilt (watermark disease) of *Salix* species. The pathogen occurs mainly in the xylem vessels of the host plant. Infected willows show wilted, dried, brown-colored leaves and a watery, transparent color of the wood. Occasionally the whole tree is killed.

   The mol% G + C of the DNA is: 51.3–51.5 (T_m, Bd.).

   Type strain: ATCC 15712, ICMP 1587, LMG 2698, NCPPB 447.

   GenBank accession number (16S rRNA): U80210 and Z96097.

2. **Brenneria alni** (Surico, Mugnai, Pastorelli, Giovannetti and Stead 1996) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 395) (*Erwinia alni* Surico, Mugnai, Pastorelli, Giovannetti and Stead 1996, 725.)

TABLE BXII.γ.209. Additional reactions for *Brenneria* species[a]

Characteristic	1. *B. salicis*	2. *B. alni*	3. *B. nigrifluens*	4. *B. paradisiaca*	5. *B. quercina*	6. *B. rubrifaciens*
Growth in 5% NaCl	nd	+	+	nd	+	nd
Growth at 37°C	nd	+	+	nd	+	nd
$NO_3^- \rightarrow NO_2^-$	nd	−	−	nd	−	nd
H_2S from cysteine	nd	+	+	nd	+	nd
Acetoin	nd	+	+	nd	+	nd
Oxidase	−	−	−	−	−	−
Catalase	+	+	+	+	+	+
Arginine dihydrolase	−	−	−	−	−	−
Caseinase	−	−	−	−	−	−
Arginine decarboxylase	−	−	−	−	−	−
Aspartase	+	nd	+	nd	nd	nd
Gelatinase	nd	−	−	nd	−	nd
Lysine decarboxylase	−	−	−	−	−	−
Ornithine decarboxylase	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	nd	−	−
Starch hydrolase	−	−	−	−	−	−
Acid production from:						
N-Acetylglucosamine	+	nd	+	+	+	+
Adonitol	−		−	−	−	−
Amygdalin	+		+	nd	+	+
Arabitol	−	nd	−	−	−	−
Arbutine	+	nd	+	+	+	+
Cellobiose	nd	nd	nd	nd	−	−
Dextrin	−	nd	−	nd	−	−
Dulcitol	−		−	−	−	−
Erythritol	−	nd	−	−	−	−
Erythrose	−		nd	−	nd	nd
Fructose	+	+	+	+	+	+
DL-Fucose	−	nd	−	−	−	−
D-Galactose	+	+	+	+	+	+
Gentiobiose	nd	nd	nd	+	nd	nd
D-Glucose	+	+	+	+	+	+
Gluconate	nd	nd	nd	+	nd	nd
Glycerol	nd	+	+	nd	+	nd
Glycogen	−	nd	−	−	−	−
Inulin	−	nd	−	nd	−	−
Lactose	−		nd	−	−	−
D-Lyxose	nd	nd	nd	−	nd	nd
D-Mannitol	+	+	+	nd	+	+
D-Mannose	+	+	+	+	+	+
α-Methylmannoside	−	nd	−	−	−	−
α-D-Melezitose	−		nd	−	−	−
β-Methylglucoside	+	nd	+	+	+	+
β-Methylxyloside	−	nd	−	−	−	−
Rhamnose	+	nd	+	+	+	+
Ribose	+	nd	+	+	+	+
Salicin	+	+	+	+	+	+
Sorbose	−	nd	−	−	−	−
Starch	−	nd	−	−	−	−
Sucrose	+	+	+	+	+	+
Tagatose	−	nd	−	−	−	−
Xylitol	nd	nd	nd	−	nd	nd
Utilization of carbon sources:						
Adipate	−	nd	−	−	−	−
Adonitol	−	nd	−	nd	v	−
Amygdalin	nd	nd	−	nd	nd	nd
D-Arabinose	−	nd	nd	nd	nd	nd
D-Arabitol	−	nd	−	−	−	−
Arbutin	+	nd	+	+	+	+
Benzoate	−	nd	−	−	−	−
Betaine	−	nd	−	−	−	−
Butanate	nd	nd	−	nd	nd	nd
Butanol	−	nd	nd	−	−	−
Cellobiose	nd	nd	−	nd	−	nd
Dextrin	−	nd	−	nd	−	−
Dulcitol	−	nd	−	nd	−	−
meso-Erythritol	−	nd	−	nd	−	−
Esculin	+	nd	+	nd	+	+
Ethylene glycol	−	nd	−	nd	−	−
Fructose	+	nd	+	+	+	+
Fumarate	+	nd	+	+	+	+
D-Galactose	+	nd	+	+	+	+
Gallate	−	nd	−	−	−	−

(*continued*)

TABLE BXII.γ.209. *(cont.)*

Characteristic	1. *B. salicis*	2. *B. alni*	3. *B. nigrifluens*	4. *B. paradisiaca*	5. *B. quercina*	6. *B. rubrifaciens*
Gluconate	+	nd	+	+	+	+
Glucose	+	nd	+	+	+	+
Glucuronic acid	nd	nd	+	nd	nd	nd
L-Glutaminic acid	−	nd	nd	nd	nd	−
Glycerol	+	nd	+	+	+	+
Glycogen	−		−	nd	−	−
Glycol	−	nd	nd	nd	nd	nd
α-Ketoglutarate	nd	nd	nd	+	nd	nd
Lactose	−	nd	−	nd	−	−
Lactulose	−	nd	−	nd	nd	−
Lyxose	−	nd	−	nd	−	−
Malate	+	nd	+	+	+	+
Malonate	nd		−	−	nd	−
Malonic acid	−	nd	nd	nd	−	−
D-Mannitol	+	nd	+	+	+	+
Mannose	+	nd	+	+	+	+
Melibiose	+	nd	+	+	nd	nd
α-D-Melezitose	−	nd	−	nd	−	−
Methanol	−	nd	−	−	−	−
α-Methylglucoside	nd	nd	nd	−	nd	nd
β-Methylglucoside	+	nd	+	+	+	+
Mucate	nd	nd	nd	+	nd	nd
Naphthalene	−		−	nd	−	−
Oxalate	−	nd	−	−	−	−
Pectinic acid	−	nd	nd	nd	nd	−
Propanol	−	nd	−	nd	−	−
Propionate	−	nd	−	−	−	−
Ribose	+	nd	+	+	+	+
Salicin	+	nd	+	+	+	+
Sorbinic acid	−	nd	−	nd	−	−
Sorbose	−	nd	−	−	−	−
Starch	−	nd	−	nd	−	−
Succinate	+	nd	+	+	+	+
Sucrose	+	nd	+	nd	+	+
Tartrate	nd	nd	nd	−	nd	nd
Triacetine	nd	nd	nd	−	nd	nd
Xylitol	−	nd	−	−	−	−
Utilization of nitrogen sources:						
Adenine	nd	nd	nd	+	nd	nd
Alanine	+	nd	+	+	+	+
Allantoine	+	nd	+	+	+	+
Ammonium chloride	+	nd	+	+	+	+
Arginine	+	nd	+	+	+	+
Asparagine	+	nd	nd	nd	+	+
Asparaginic acid	+	nd	+	+	+	+
Betaine	−	nd	−	−	−	−
Carnosine	nd	nd	nd	+	nd	nd
Choline	−	nd	−	−	−	−
Citrulline	+	nd	+	+	+	+
Cysteamine	−	nd	−	−	−	−
Creatine	nd	nd	nd	+	nd	nd
Ethanolamine	nd	nd	+	nd	+	+
Glucosamine	+	nd	+	+	+	+
L-Glutaminic acid	+	nd	+	+	+	+
Glutathion	+	nd	+	+	+	+
Glycine	+	nd	+	+	+	+
Glycylglycine	+	nd	+	+	+	+
Guanine	nd	nd	nd	−	nd	nd
Histidine	+	nd	+	+	+	+
Hydroxyproline	−	nd	−	−	−	−
Isoleucine	+	nd	nd	nd	+	+
Kynureninic acid	−	nd	−	−	−	−
Leucine	+	nd	+	+	+	+
L-Methionine	+	nd	+	+	+	+
Nicotinic acid	nd	nd	nd	−	nd	nd
Octopine	nd	nd	nd	+	nd	nd
Ornithine	nd	nd	nd	+	nd	nd
Phenylalanine	+	nd	+	+	+	+
Quinolinic acid	−	nd	−	−	−	−
Sarcosine	−	nd	−	−	−	−
Spermidine	−	nd	−	−	−	−
Spermine	−	nd	−	−	−	−
Taurine	nd	nd	nd	−	nd	nd

(*continued*)

TABLE BXII.γ.209. *(cont.)*

Characteristic	1. *B. salicis*	2. *B. alni*	3. *B. nigrifluens*	4. *B. paradisiaca*	5. *B. quercina*	6. *B. rubrifaciens*
Thymine	−	nd	−	nd	−	−
Trigonelline	−	nd	−	−	−	−
L-Tryptophan	+	nd	nd	+	+	+
Tyrosine	+	nd	+	+	+	+
Ureum	+	nd	+	+	+	+
Valine	+	nd	+	+	+	+
Sensitivity toward:						
Amoxycillin	nd	nd	+	+	+	+
Ampicillin	nd	nd	+	+	+	+
Bacitracin	−	nd	−	nd	−	−
Carbenicillin	+	nd	+	+	+	+
Cephalexin	+	nd	+	+	+	+
Cephaloridine	+	nd	+	+	+	+
Cephalotine	+	nd	+	+	nd	+
Chloramphenicol	+	nd	+	+	+	+
Cloxacyclin	−	nd	−	nd	−	−
Colistine sulfate	−	nd	−	nd	−	−
Doxycylin	+	nd	+	nd	+	+
Erythromycin	−	nd	−	nd	−	−
Framycetine	+	nd	+	+	nd	+
Furazolidone	+	nd	+	+	+	+
Fusidinic acid	−	nd	−	−	−	−
Gentamicin	−	nd	−	nd	−	−
Kanamycin	+	nd	nd	+	v	nd
Lincomycin	−	nd	−	nd	nd	−
Methicillin	−	nd	−	−	−	−
Nalidixinic acid	+	nd	+	+	+	+
Neomycin	nd	nd	−	nd	−	nd
Nitrofurantoin	+	nd	+	nd	+	+
Oxytetracycline	+	nd	+	+	+	+
Penicillin	nd	nd	+	nd	+	+
Polymyxin	nd	nd	−	nd	nd	−
Spectinomycin	−	nd	−	nd	nd	nd
Sulfafurazol	−	nd	−	−	−	−
Novobiocine	nd	nd	+	nd	nd	nd
Tetracycline	+	nd	+	+	+	+

[a]For symbols see standard definitions; nd, not determined.

al' ni. L. fem. n. *alnus* alder; L. gen. n. *alni* of alder, referring to the plant from which the organism was first isolated.

The characteristics are as given for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

Abundant growth occurs in nutrient broth and on nutrient agar, YDC (0.5% yeast extract, 1% dextrose, 3% $CaCO_3$), Miller–Schroth medium. Colonies on NSA (nutrient agar containing 5% sucrose) are umbonate with translucent margins, whitish, and 2–2.5 mm in diameter after 5 days at 27°C.

Causes bark canker disease of alder (*Alnus* sp.). At first, diseased alder plants have small necrotic cankers in the bark of the trunk and in the bark of branches, twigs, and suckers. These cankers are slightly sunken, dark brown, and irregularly circular and appear to be water soaked. They usually develop at lenticels or are localized at nodes with dead twigs or on leaf scars. As the infection progresses, the necrosis spreads laterally, becoming deeper and reaching the cambium and sometimes the first layers of wood, which then turn brown. Cankers may eventually girdle and kill a branch or tree.

The mol% G + C of the DNA is: 50.1–50.7 (T_m).
Type strain: ICMP 12481, NCPPB 3934.
GenBank accession number (16S rRNA): AJ223468.

3. **Brenneria nigrifluens** (Wilson, Starr and Berger 1957) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 395) (*Erwinia nigrifluens* Wilson, Starr and Berger 1957, 673.)
ni.gri.flu' ens. L. adj. *niger* nigra black; L. v. *fluo* flow; M.L. part. adj. *nigrifluens* black flowing.

The characteristics are as described for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

Colonies on Bacto-EMB (Difco) agar are dark violet with a green metallic sheen. Craters form around colonies on the polypectate medium (1 liter nearly boiling distilled water, 1 ml of bromothymol blue, 6 ml of 10% $CaCl_2 \cdot H_2O$, 2 g of sodium polypectate) of Hildebrand (1971). Growth media should contain yeast extract and should be at pH 7–8.

Causes a bark necrosis of the Persian walnut (*Juglans regia*). The disease is visible as dark brown necrotic areas on the trunks or large branches. The lesions extend well beyond the discolored bark. Exudates may be produced. The lesions are superficial, but may penetrate enough to kill the phloem.

The mol% G + C of the DNA is: 56.1 (T_m, Bd).
Type strain: ATCC 13028, ICMP 1578, LMG 2694.
GenBank accession number (16S rRNA): U80203, Z96095.

4. **Brenneria paradisiaca** (Fernández-Borrero and López-Duque 1970) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publica-

tion: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 396) (*Erwinia paradisiaca* Fernández-Borrero and López-Duque 1970, 22.)
pa.ra.di.si.a' ca. M.L. n. generic name *Musa paradisiaca* banana.

The characteristics are as given for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

Causes rhizome rot of roots and soft rot of green fruit of *Musa paradisiaca*.

The mol% G + C of the DNA is: 54.7 (T_m, Bd).
Type strain: ATCC 33242, LMG 2542, NCPPB 2511.
GenBank accession number (16S rRNA): Z96096.

5. **Brenneria quercina** (Hildebrand and Schroth 1967) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 395) (*Erwinia quercina* Hildebrand and Schroth 1967, 253.)
quer.ci' na. L. n. *quercus* oak; L. suff. *ina* belonging to; M.L. part. adj. *quercina* oak-belonging.

The characteristics are as given for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

Growth on PGPC agar (60 g homogenized potato, 10 g glucose, 10 g peptone, 1 g $CaCO_3$, 1 liter distilled water) is luxuriant, and, after 24 h, colonies are white, circular, and raised with entire margins. Craters form around colonies on polypectate gel of Hildebrand (1971), but no pectolytic activity was observed in potato or carrot tissue.

Small amounts of gas are produced (possibly from peptone) in a glucose peptone medium and in PGPC.

Causes copious oozing of sap from acorns ("drippy nut") and shoot blight of oak: *Quercus agrifolia* and *Q. wislizeni*. Acorns may rot somewhat, and cups may ooze after nutfall. Oozing takes place in late summer when temperatures average about 29°C. Superficially rots onion (but not potato) slices and induces profuse lateral root development in 3–4 days on slices of carrot, turnip, or beet.

The mol% G + C of the DNA is: from 54.6–55.1 (T_m, Bd).
Type strain: ATCC 29281, ICMP 1845, LMG 2724, NCPPB 1852.
GenBank accession number (16S rRNA): AJ223469.

6. **Brenneria rubrifaciens** (Wilson, Zeitoun and Fredrickson 1967) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 395) (*Erwinia rubrifaciens* Wilson, Zeitoun and Fredrickson 1967,621.)
rub.ri.fac' i.ens. L. adj. *ruber* red; L. v. *facio* make; M.L. part. adj. *rubrifaciens* red-producing.

The characteristics are as given for the genus and as listed in Tables BXII.γ.208 and BXII.γ.209.

Produces a pink soluble pigment when grown on yeast extract glucose chalk agar (YDC, 0.5% yeast extract, 1% dextrose, 3% calcium carbonate (Dye, 1968). The structure of a 3-(3-carboxymethyl-4,5-dihydro-4,5-dioxo-2-pyrrolyl)-4,5,6-trihydroxy-pyridine-2-acetic acid (rubrifacine) is suggested (Feistner et al., 1984) as the main component of the pigment.

Grows poorly on nutrient agar, but well on YDC on which colonies are cream to yellow, low convex, smooth, shining with entire margins. Craters form around colonies on the polypectate gel B and C of Hildebrand (1971), but no pectolytic activity was observed on vegetable tissue.

Causes a phloem necrosis of Persian walnut trees (*Juglans regia*). Dark brown to black necrotic streaks from along the inner bark and outer sapwood. In places, the necrosis develops outward to the periderm, which breaks, and a dark exudate emerges.

The mol% G + C of the DNA is: 52.0–52.6 (T_m, Bd).
Type strain: ATCC 29291, ICMP 1915, LMG 2709, NCPPB 2020.
GenBank accession number (16S rRNA): U80207 and Z96098.

Genus V. **Buchnera** *Munson, Baumann and Kinsey 1991b, 566[VP]*

PAUL BAUMANN, LINDA BAUMANN AND NANCY A. MORAN

Buch.ne' ra. M.L. fem. n. *Buchnera* named for Paul Buchner, German biologist who made extensive contributions to the study of endosymbiosis.

Round or slightly oval cells, 2–5 μm in diameter. **Gram-negative** cell wall, lack flagella. Cells divide by binary fission. Do not form resting stages or endospores. **Found in bacteriocytes** of aphids in vesicles derived from the host membranes. Capable of respiration. **Cannot be cultivated** outside the aphid host. Essential for the survival of the aphid.

The mol% G + C of the DNA is: 26–27.

Type species: **Buchnera aphidicola** Munson, Baumann and Kinsey 1991b, 567.

FURTHER DESCRIPTIVE INFORMATION

Cell structure and life cycle During their reproductive phase most aphids contain within their body cavity a bilobed structure called a bacteriome consisting of 60–90 polyploid cells called bacteriocytes (Douglas and Dixon, 1987). These cells are filled with host-derived vesicles (symbiosomes) containing *Buchnera* (Fig. BXII.γ.194) (Hinde 1971b; Griffiths and Beck, 1973; McLean and Houk, 1973; Akhtar and van Emden, 1994). This organism is spherical or oval in shape having a diameter of 2–5 μm and a cell wall resembling that of Gram-negative bacteria (Fig. BXII.γ.195). A thin line indicative of the peptidoglycan layer has been detected between the two unit membranes (Houk et al., 1977). The presence of peptidoglycan is also indicated by chemical analysis and by the alteration of cell wall structure observed upon addition of penicillin to the aphid diet (Griffiths and Beck, 1974; Houk et al., 1977).

In their most active reproductive stage, aphids are females that reproduce by parthenogenesis. The embryos develop within the mother, which gives birth to live young (Blackman, 1987). A representative aphid, *Schizaphis graminum*, when born weighs 24 mg and contains 2×10^5 cells of *Buchnera*. In 9–10 d, it reaches its maximum weight of 540 mg and contains 5.6×10^6 endo-

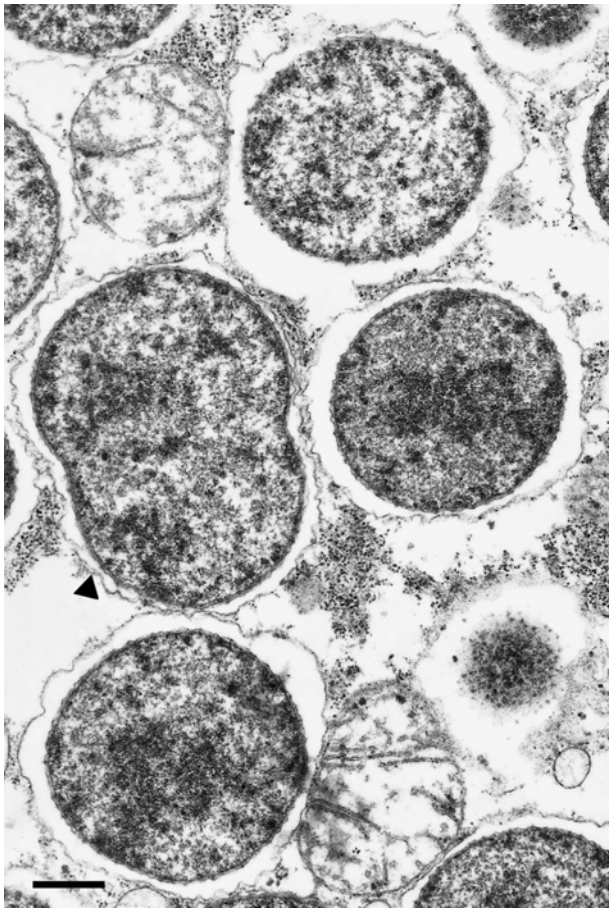


FIGURE BXII.γ.194. Electron micrograph of *Buchnera* within aphid bacteriocytes. *Arrow* indicates host-derived vesicle membrane. Bar = 1 μm. (Printed with permission of M. Kinsey and D.L. McLean.)

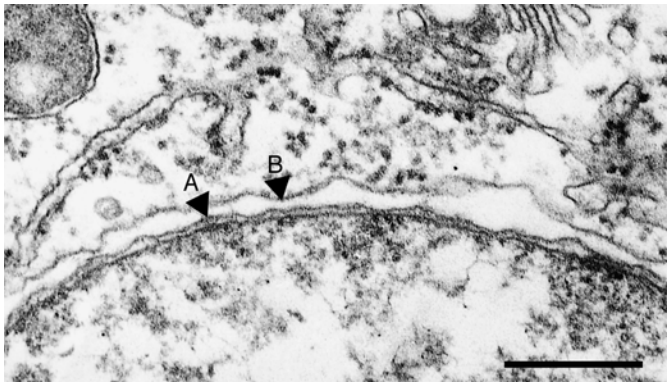


FIGURE BXII.γ.195. Electron micrograph of *Buchnera* showing the Gram-negative cell wall (*A*) and the vesicle membrane (*B*). Bar = 0.5 μm. (Printed with permission of M. Kinsey and D.L. McLean.)

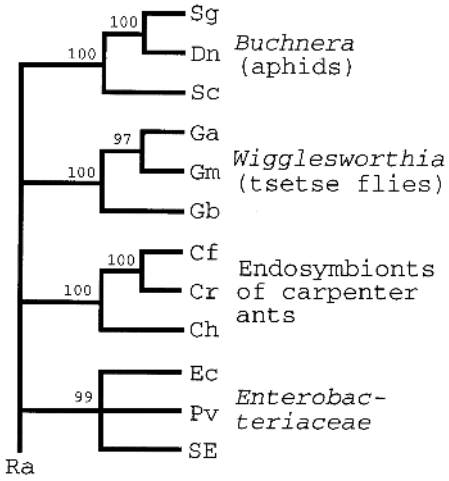


FIGURE BXII.γ.196. Phylogenetic trees resulting from parsimony analyses (D.L. Swofford, 1993, PAUP 3.1.1, Champaign, IL) using 16S rDNA nucleotide sequences (1189 sites) of *Buchnera* and other closely related insect endosymbionts as well as free-living bacteria. Numbers above nodes are bootstrap percentages from parsimony searches (1000 replicates). Data for *Wigglesworthia glossinidia* from Aksoy (1995b); the following abbreviations designate the insect hosts (in parentheses): *Ga* (*Glossina austeni*), *Gm* (*G. morsitans*), *Gb* (*G. brevipalpis*). Data for carpenter ant endosymbionts from Schröder et al. (1996); hosts: *Cf* (*Camponotus floridanus*), *Cr* (*C. rufipes*), *Ch* (*C. herculeanus*); *SE* (secondary endosymbiont of the aphid *Acyrthosiphon pisum*); *Ra* (*Ruminobacter amylophilus*).

symbionts (Baumann and Baumann, 1994). The increase in the total number of *Buchnera* parallels the increase in the weight of the aphid. The endosymbionts are partitioned between the maternal and the embryonic bacteriocytes. In mature aphids, most of the endosymbionts are found in the embryos (Humphreys and Douglas, 1997). Halfway through the reproductive period the number of maternal bacteriocytes undergoes a sharp decrease (Douglas and Dixon, 1987) and there is degradation of the endosymbionts (Hinde, 1971a; Griffiths and Beck, 1973).

Aphids may also form sexual forms; the females deposit eggs that overwinter and hatch in the spring. *Buchnera* is transmitted maternally to the embryos and the eggs. The mechanism of transmission is complex and has not been extensively studied (Hinde, 1971a; Blackman, 1987; Brough and Dixon, 1990).

Phylogenetic analyses Based on 16S rDNA phylogeny, *Buchnera* is a lineage within the *Gammaproteobacteria* (Unterman et al., 1989; Munson et al., 1991a; Moran et al., 1993; van Ham et al., 1997). The closest known organisms (Fig. BXII.γ.196) are the nonculturable *Wigglesworthia glossinidia* (endosymbionts of tsetse flies) and endosymbionts of carpenter ants as well as members of the *Enterobacteriaceae* (Aksoy, 1995b; Aksoy et al., 1995; Schröder et al., 1996). These organisms constitute four separate lineages and their relationship to each other is not clearly resolved based on 16S rDNA.

Fig. BXII.γ.197 presents the results of a phylogenetic analysis primarily restricted to *Buchnera*. Most of the characterized endosymbionts are from aphids belonging to the family *Aphididae*. Based on 16S rDNA gene sequences (Fig. BXII.γ.197a), *Buchnera* forms one cluster within which a subcluster of endosymbionts from aphids of *Aphididae* is clearly differentiated. An additional cluster consists of endosymbionts from the aphids *Melaphis rhois* (Mr) and *Schlechtendalia chinensis* (Sc), both of which are members of the tribe Melaphidini in the family *Pemphigidae*. Relationships between the remaining endosymbionts from this family as well as from two other aphid families are not clearly resolved using 16S rDNA gene sequences. Using a portion of the chromosomal *trpB*, relationships among endosymbionts of some members of the *Aphididae* and especially within the aphid genus *Uro-*

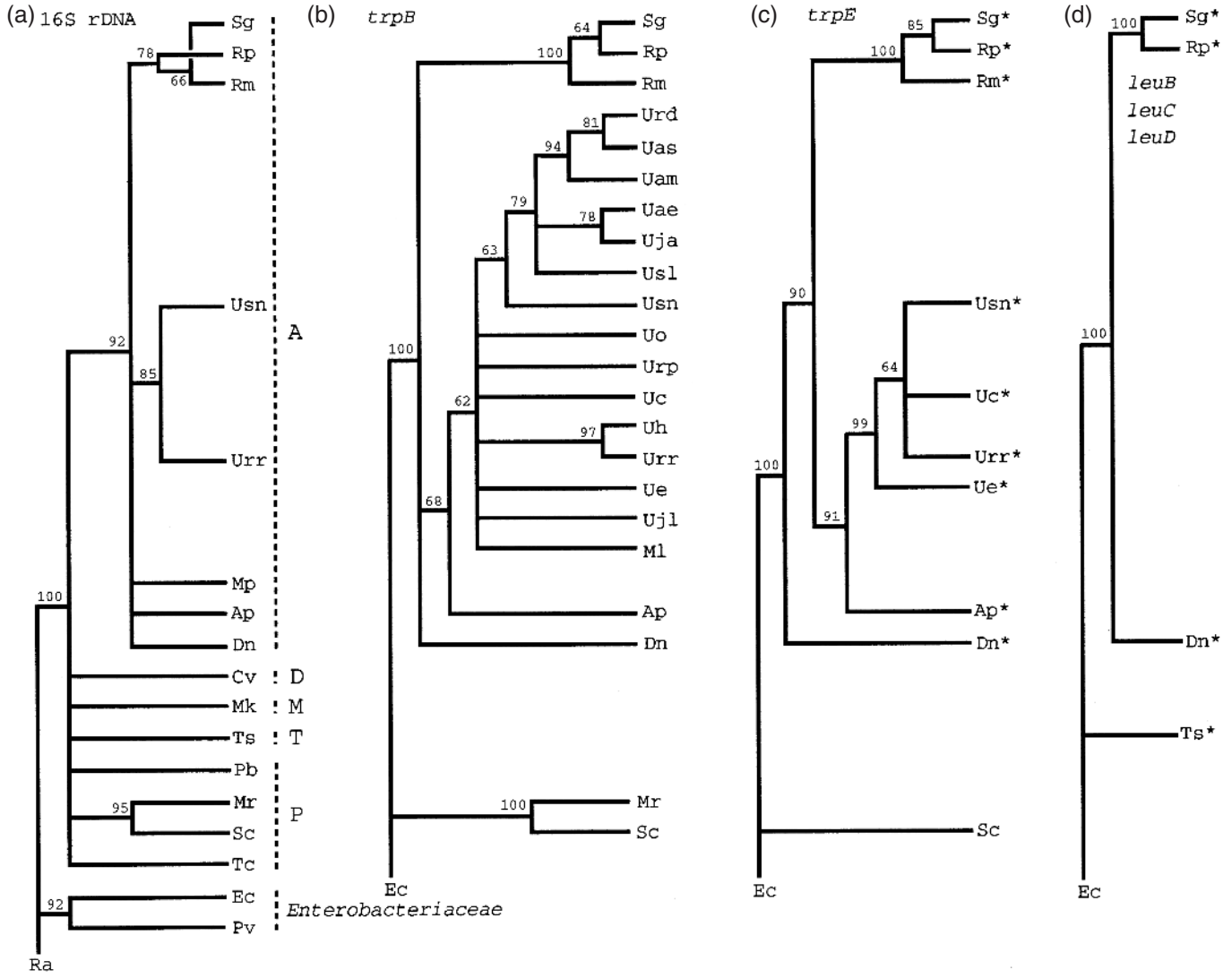


FIGURE BXII.γ.197. Phylogenetic trees resulting from parsimony analyses (D.L. Swofford, 1993, PAUP 3.1.1, Champaign, IL) using *Buchnera* (*a*) 16S rDNA (1475 sites), (*b*) *trpB* (679 sites), (*c*) *trpE* (1623 sites), and (*d*) *leuB*, *leuC*, and *leuD* (total 3140 sites). *, plasmid-associated genes; numbers above nodes are bootstrap percentages from parsimony searches (1000 replicates). Abbreviations designate insect hosts in the order from top to bottom (given in parentheses): *Sg* (*Schizaphis graminum*), *Rp* (*Rhopalosiphum padi*), *Rm* (*R. maidis*), *Urd* (*Uroleucon rudbeckiae*), *Uas* (*U. astronomus*), *Uam* (*U. ambrosiae*), *Ua*e (*U. aeneum*), *Uja* (*U. jaceiae*), *Usl* (*U. solidaginis*), *Usn* (*U. sonchi*), *Uo* (*U. obscurum*), *Urp* (*U. rapunculoidis*), *Uc* (*U. caligatum*), *Uh* (*U. helianthicola*), *Urr* (*U. rurale*), *Ue* (*U. erigeronense*), *Ujl* (*U. jaceicola*), *Ml* (*Macrosiphoniella ludovicianae*), *Mp* (*Myzus persicae*), *Ap* (*Acyrthosiphon pisum*), *Dn* (*Diuraphis noxia*), *Cv* (*Chaitophorus viminalis*), *Mk* (*Mindarus kinseyi*), *Ts* (*Thelaxes suberi*), *Pb* (*Pemphigus betae*), *Mr* (*Melaphis rhois*), *Sc* (*Schlechtendalia chinensis*), *Tc* (*Tetraneura caerulescens*). Species of the *Enterobacteriaceae*: *Ec* (*E. coli*), *Pv* (*P. vulgaris*). Dashed lines in 4a designate aphid species within one family: *A*, *Aphididae*; *D*, *Drepanosiphidae*; *M*, *Mindaridae*; *T*, *Thelaxidae*; *P*, *Pemphigidae*. Data for *Ts* and *Tc* in 4a and 4d from van Ham et al. (1997); for *Rp* in 4d from Bracho et al. (1995); for other sources of data for *Buchnera* see text.

leucon could be resolved (Fig. BXII.γ.197b). These relationships are in broad agreement with more limited studies involving *Buchnera* plasmid-associated *trpE* and plasmid-associated *leuBCD* (Fig. BXII.γ.197c and d) (Bracho et al., 1995; Rouhbakhsh et al., 1996, 1997; Baumann et al., 1997, 1999; van Ham et al., 1997). In addition, the phylogenetic relationships based on *trpB* agree with the phylogeny reconstructed for the same species of *Uroleucon* using nuclear and mitochondrial sequences from the aphids (Moran et al., 1999). This pattern of congruence of phylogenies derived from chromosomal genes, plasmid genes, and host genes is strong support for a completely vertical mode of evolution, with no transfer of either bacteria or plasmids among lineages of hosts (Moran and Baumann, 1994). An implication of the congruence is that bacterial and host ancestors representing corresponding nodes on the phylogenies occurred contemporaneously, allowing dates inferred from fossil aphids to be extended to ancestral *Buchnera* (Moran et al., 1993). Phylogenetic analysis and the rate of 16S rDNA change inferred from fossil aphids has suggested that the *Buchnera*–aphid association is the result of a single infection of an aphid ancestor that occurred 150–250 million years ago. In *Buchnera* the rate of 16S rDNA evolutionary change is about twice that of free-living bacteria and about 36 times greater than in homologous regions of the host 18S rDNA (Moran, 1996; Moran et al., 1995). The increased rate of change in endosymbionts compared to free-living bacteria extends to genes other than 16S rDNA (Moran, 1996).

Nutrition and metabolism Insects, like other animals, are thought to require preformed ten essential amino acids (Dadd, 1985; Douglas, 1998). Plant sap (phloem), the diet of aphids, is rich in carbohydrates but is deficient in nitrogenous compounds (Dadd, 1985; Sandström and Pettersson, 1994). It has been thought that one of the functions of *Buchnera* is the synthesis of the essential amino acids and their provision to the aphid host (Dadd, 1985; Douglas, 1998). Some species of aphids are able to grow on synthetic diets even in the absence of some of the essential amino acids. The incorporation of antibiotics into such diets results in the elimination of the endosymbionts and the failure of the aphid to grow and reproduce. There is some sparing effect when essential amino acids are included in the antibiotic-containing diet. These results have been interpreted as being consistent with the synthesis of essential amino acids by the endosymbionts (Douglas, 1998). The most detailed experiments involve the essential amino acid tryptophan. Douglas and Prosser (1992) have shown there is a sparing effect of tryptophan on aphid growth in chlortetracycline-containing synthetic diet. In addition, these investigators detected the enzyme tryptophan synthase in *Buchnera*. Activity was absent in aphids treated with chlortetracycline that lacked the endosymbionts.

Using synthetic diets containing radiolabeled amino acids, it has been shown that the synthesis of the essential amino acids arginine, threonine, isoleucine, and lysine was eliminated by the inclusion of rifampicin in the diet (Liadouze et al., 1996). Similarly, experiments using radiolabeled sulfate showed that endosymbionts reduce sulfur and incorporate it into methionine and cysteine, which are made available to aphid tissue (Douglas, 1988). Sasaki et al. (1991) showed that treatment of aphids with rifampicin eliminated the incorporation of dietary [^{15}N]-glutamine into the essential amino acids arginine, histidine, isoleucine and/or leucine, phenylalanine, threonine, and valine.

Glutamine is one of the predominant amino acids in phloem (Sasaki et al., 1990; Sandström and Pettersson, 1994). Sasaki and Ishikawa (1995) have found that it is the predominant amino acid in aphid hemolymph. These investigators have shown that bacteriocytes take up glutamine and convert it to glutamate. Subsequently glutamate is taken up by *Buchnera*. The isolated endosymbionts incorporate the nitrogen from this compound into the essential amino acids isoleucine, leucine, valine, and phenylalanine as well as into a number of other nonessential amino acids.

Whitehead and Douglas (1993) isolated vesicles containing *Buchnera* (symbiosomes). The symbiosomes readily took up acetic, glutamic, and aspartic acid as well as tricarboxylic acid cycle intermediates and oxidized them to CO_2. Oxygen consumption was also detected and was greatly reduced by KCN. These results indicate a respiratory metabolism.

Genetics *Buchnera* contains a single copy of the genes encoding for rRNAs that are organized into two transcription units consisting of 1) 16S rRNA (GenBank accession number M63246) and 2) tRNA ^{Glu}–23S rRNA–5S rRNA (GenBank accession number U09230) (Baumann et al., 1995). A single copy of these genes is consistent with the slow growth rate of the endosymbiont (Baumann et al., 1995). Upstream of the rRNA genes are sequences that are readily recognized as -35 and -10 regions of a putative promoter.

The genome size of *Buchnera* from the aphid *Acyrthosiphon pisum* has been found to be 657 kb (Charles and Ishikawa, 1999). Approximately 113 kb of the genome of *Buchnera* from the aphid *S. graminum*, 12 kb from the endosymbiont of *Diuraphis noxia*, 11 kb from the endosymbiont of *S. chinensis*, and 6 kb from the endosymbiont of *A. pisum* have been sequenced (Ohtaka et al., 1992; Baumann et al., 1995; Sato and Ishikawa, 1997b; Baumann and Baumann, 1998; Clark et al., 1998a, 1998b; Thao and Baumann, 1998). The gene content obtained from the nucleotide sequence is an indication of the metabolic capacity of an organism. In *Buchnera* from *S. graminum* 109 open reading frames were detected. Of these, 89 corresponded to known genes and the remaining 20 had similarities to open reading frames on the *E. coli* genome, which have no known function (Blattner et al., 1997). *Buchnera* contains *dnaA*, encoding a protein that initiates bidirectional chromosome replication, and *ftsZ*, encoding a protein involved in septum formation during cell division (Lai and Baumann, 1992; Baumann and Baumann, 1998). Among other genes that were found are those encoding proteins for peptidoglycan synthesis, cell division, DNA replication, DNA transcription, ribosomal proteins, amino acid tRNA synthases, ATP synthase, electron transport, protein secretion, and glycolysis. In addition, genes for three tRNAs were detected. Genes encoding homologs of proteins involved in the *E. coli* heat shock response (*groEL*, *groES*, *htrA*, *dnaK*, *dnaJ*) and the cold shock response (*hscA*, *hscB*) were also detected (Ohtaka et al., 1992; Hassan et al., 1996; Sato and Ishikawa, 1997b; Clark et al., 1998b). In addition, some of the genes encoding enzymes for the biosynthesis of aromatic amino acids (shikimate pathway, tryptophan branch), branched chain amino acids (isoleucine, valine, leucine), lysine, cysteine, and serine, as well as genes for the complete pathway of histidine biosynthesis, were also found. The presence of genes for enzymes of amino acid biosynthesis is in marked contrast to such fastidious organisms as *Mycoplasma genitalium* and *Borrelia burgdorferi* in which the genes encoding enzymes of amino acid biosynthesis were absent (Fraser et al., 1995, 1997). Retention of amino acid biosynthetic genes may reflect the role of these pathways in the mutualistic association with the host aphids.

The overproduction of essential amino acids for the aphid host may involve modifications of some mechanisms that regulate the levels of biosynthetic enzymes (Baumann et al., 1995). One possible modification is an increase in the level of a key regulated enzyme by gene amplification. This appears to be the case in the tryptophan biosynthetic pathway of rapidly growing aphids in which the genes for the enzyme anthranilate synthase (*trpEG*) are present as tandem repeats on plasmids. In the case of *Buchnera* from *S. graminum*, *trpEG* is present as four tandem repeats that constitute a plasmid of 14.3 kb (Lai et al., 1994). The remaining genes of the tryptophan biosynthetic pathway (*trpDC[F]BA*) are chromosomal (Munson and Baumann, 1993). There are three to four copies of the *trpEG*-containing plasmid for each of the chromosomal genes. Amplification of *trpEG* is widespread in *Buchnera* of the family *Aphididae*, which is composed of rapidly growing aphids (Fig. BXII.γ.197c) (Lai et al., 1994; Rouhbakhsh et al., 1996, 1997). In each case, the region upstream of *trpE* contains two or more copies of a nine-nucleotide sequence known as a DnaA box. Since the DnaA protein binds to this sequence and initiates DNA replication, this region may be a putative origin of plasmid replication. In the slow-growing aphid *Schlechtendalia chinensis*, *trpEG* is present as one copy on the *Buchnera* chromosome (Lai et al., 1995). Curiously, the *trpEG*-containing plasmids of *Buchnera* from *D. noxia* and from *U. sonchi* contain an intact copy of *trpEG* as well as numerous copies of *trpEG* pseudogenes (Lai et al., 1996; Baumann et al., 1997). There is currently no satisfactory explanation for this observation.

An independent case in which genes for amino acid biosyn-

thesis are plasmid-borne involves the leucine biosynthetic pathway. In *Buchnera* from several aphids, all of the enzymes of leucine biosynthesis (*leuABCD*) are on plasmids (pLeu) of about 8 kb (Bracho et al., 1995; van Ham et al., 1997; Baumann et al., 1999). In *Buchnera* from the aphid *S. graminum* there are about 23 copies of pLeu per endosymbiont chromosomal gene (Thao et al., 1998). Aphids from which pLeu has been sequenced are given in Fig. BXII.γ.197d. These plasmids have no similarity to the *trpEG*-containing plasmids and lack DnaA boxes. Instead they contain open reading frames (*repA1*, *repA2*) that correspond to putative proteins related to RepA of the IncFII incompatibility group plasmids, which is involved in plasmid DNA replication (Bracho et al., 1995). Also encoded on pLeu is a conserved open reading frame of unknown function. A most remarkable finding is the presence of a 1.7-kb plasmid in *Buchnera* from the aphid *Tetraneura caerulescens*, which contains only *repA1* and an unidentified open reading frame (van Ham et al., 1997). It is possible that this plasmid represents a minimal-size replicon that under the right selection can be used for the amplification of other chromosomal genes.

GroEL overproduction In *Buchnera*, the chaperonin GroEL constitutes a major fraction of the total protein (Sato and Ishikawa, 1997b). In addition, GroEL is detected in the aphid hemolymph (van den Heuvel et al., 1994). Overproduction of GroEL is a characteristic of endosymbionts and pathogens in the intracellular environment (Baumann et al., 1995; Hogenhout et al., 1998). GroEL has been localized in maternal and embryonic endosymbionts by immunohistochemistry (Fukatsu and Ishikawa, 1992b). Electron micrographs indicate that the purified *Buchnera* GroEL has the characteristic double-ring appearance observed with the *E. coli* protein (Hara and Ishikawa, 1990; Filichkin et al., 1997). In addition, it has ATPase activity and in the presence of *E. coli* GroES and ATP could reconstitute denatured *Rhodospirillum rubrum* ribulose-1,5-biphosphate carboxylase (Kakeda and Ishikawa, 1991). *Buchnera* GroEL is able to complement *E. coli* mutants (Ohtaka et al., 1992).

The *Buchnera groESL* operon organization resembles that of *E. coli* (Ohtaka et al., 1992; Hassan et al., 1996; Hogenhout et al., 1998). Upstream of *groES* are nucleotide sequences characteristic of the -35 and -10 regions of σ^{32} promoters. A message of 2.1 kb (containing both *groES* and *groEL*) is made by the endosymbiont using only this promoter (Sato and Ishikawa, 1997a). It is not understood why GroES is barely detectable in the endosymbiont, in contrast to the high quantities of GroEL (Kakeda and Ishikawa, 1991; Fukatsu and Ishikawa, 1993). The genes for σ^{32} (*rpoH*) as well as *dnaKJ* have been cloned and sequenced (Sato and Ishikawa, 1997a, b). The latter are also transcribed solely from a σ^{32} promoter. In *E. coli* as well as other organisms, transcription of the *groESL* operon and the *dnaKJ* operon are in part of the σ^{32} regulon and their synthesis is increased by heat shock (Gross, 1996). It would appear that this mode of regulation is modified in *Buchnera* (Sato and Ishikawa, 1997a, b). Synthesis of *groESL* and *dnaKJ* messenger RNA is constitutive and is not increased by heat shock. This conclusion is supported by the observation that there is no increase in the level of total GroEL in aphids shifted from a temperature of 23°C to 33°C for a period of 1 d (Baumann et al., 1996).

Pathogenicity Aphids are major pests of agricultural plants (Blackman and Eastop, 1984). Although large populations of aphids can cause plant debilitation due to nutrient consumption, perhaps the major economic effect of aphids on agriculture is due to their transmission of plant viruses. Recently, *Buchnera*-derived GroEL has been implicated in the survival of luteoviruses in the hemolymph (van den Heuvel et al., 1994; Filichkin et al., 1997; Hogenhout et al., 1998). These viruses replicate in the plant and are ingested by aphids when they feed on phloem sap. Subsequently they are transported from the digestive tract into the hemolymph and from there into the salivary gland for transmission to plants via salivary secretions. The viruses are retained in an infective form (without replication) in the hemolymph throughout the life span of the aphid. There is evidence that the GroEL that is found in the hemolymph coats the virus particles and protects them from host defenses. A region in *Buchnera* GroEL has been identified that is essential for binding to the virus (Hogenhout et al., 1998), and similarly a portion of a viral capsid protein has been identified as the region to which the endosymbiont GroEL binds (van den Heuvel et al., 1997).

Antibiotic and drug sensitivity A variety of antibiotics have been used to eliminate endosymbionts, resulting in a reduction of weight gain of the aphid and the loss of reproductive potential. These antibiotics include chloramphenicol, chlortetracyline, neomycin, penicillin, rifampicin, and streptomycin.

Additional symbionts Many species of aphids also contain rod-shaped procaryotic endosymbionts designated as the secondary (S)-endosymbionts (Buchner, 1965; Houk and Griffiths, 1980; Fukatsu and Ishikawa, 1993). These organisms are usually absent from bacteriocytes and are found in vesicles within the sheath cells that surround the bacteriome. The S-endosymbiont from the aphid *A. pisum* is a member of the *Enterobacteriaceae* (Unterman et al., 1989). In this organism, the 16S rDNA is upstream of 23S rDNA (Unterman et al., 1989; Unterman and Baumann, 1990). The S-endosymbiont of *A. pisum* is dispensable (Chen and Purcell, 1997) since strains lacking this endosymbiont have been found in nature. The S-endosymbiont can be introduced into such strains by injection of hemolymph from strains of *A. pisum* that contain the S-endosymbiont. Some aphids may also contain a rod-shaped rickettsia closely related to *Rickettsia bellii* (Chen et al., 1996).

Although most species of aphids appear to contain bacteriocytes with *Buchnera*, some species of the tribe Cerataphidini lack both (Fukatsu and Ishikawa, 1992a; Fukatsu et al., 1994). These aphids instead contain, in their body cavity, a yeast-like symbiont that based on its 18S rDNA was found to belong to the subphylum Ascomycotine of the class Pyrenomycetes (Fukatsu and Ishikawa, 1996). These symbionts were related to those of planthoppers.

Enrichment and Isolation Procedures

Endosymbiont-enriched preparations have been obtained using methods previously described (Ishikawa, 1982; Harrison et al., 1989; Sasaki and Ishikawa, 1995). The endosymbionts require high osmolarity for the retention of their structure. The best criteria of purity have involved examination of the purified preparations by electron microscopy, which also allows determination of whether the endosymbionts are still within host-derived vesicles.

Both the aphids and the endosymbionts have a similar mol% G + C DNA content (Ishikawa, 1987; Unterman and Baumann, 1990), and consequently endosymbiont DNA cannot be separated from host DNA by CsCl density gradient centrifugation. For the routine purification of *Buchnera* from *S. graminum*, to obtain starting material for the preparation of endosymbiont-

enriched DNA, the method of Sasaki and Ishikawa (1995) has been used. All of the reagents and equipment are kept on ice and the procedures are performed as rapidly as possible. Approximately 2–3 g (wet weight) aphids are transferred to a 1.5 cm diameter tissue grinder. Ten milliliters of buffer A[1] is added and the aphids are ground with a loose-fitting plunger for 5 min and the preparation passed through a double layer of a nylon mesh to remove large particulate material. The filtrate is brought to a volume of about 100 ml with buffer A and then quickly passed through a 100-µm nylon filter (Spectrum Medical Industries, Inc., Houston, Texas, USA), followed by filtration through 20-µm and 10-µm nylon filters. Only slight vacuum pressure is applied during the last two filtration steps. The volume is made up to approximately 120 ml with buffer A and 30-ml aliquots are centrifuged in a swinging bucket rotor for 6 min at 1500 × g. The pellets are gently resuspended in 1.25 ml of buffer A and used for DNA purification (Unterman et al., 1989; Munson et al., 1991a). The DNA obtained from such preparations is endosymbiont-enriched 13- to 15-fold relative to the DNA obtained from the whole aphid (Baumann et al., 1997). Further purification of the endosymbiont preparation can be obtained by centrifugation through 10–90% Percoll gradients (Pharmacia Biotech, Uppsala, Sweden) as described by Ishikawa (1982).

Maintenance Procedures

Established lines of *S. graminum*, the aphid that contains the type strain of *B. aphidicola*, can be readily grown on wheat or barley at 23–24°C with 1500 foot candles illumination and a photoperiod of 16 h light and 8 h dark. Seeds are planted in rich soil, and, after 5–6 d, the seedlings are inoculated with aphids. The inoculum is obtained by cutting aphid-infested plants close to the roots and shaking them over the seedlings to dislodge the aphids. New seedlings are inoculated every 7–10 d.

Procedures for Testing Special Characters

Buchnera is somewhat unusual in that its rRNA genes are organized into two transcription units (Baumann et al., 1995). Consequently, 16S rDNA is not upstream of 23S rDNA. In eight strains of *Buchnera*, encompassing the diversity presented in Fig. BXII.γ.197a, *aroE* has been found to be upstream of 23S rDNA (Rouhbakhsh and Baumann, 1995). This property has been used for the identification of *Buchnera* using oligonucleotide primers complementary to *aroE* and 16S rDNA and PCR (Rouhbakhsh et al., 1994). This and other PCR-based identification methods are discussed by Rouhbakhsh et al. (1994). It should be noted that the S-endosymbiont found in some aphids has 16S rDNA upstream of the 23S rDNA (Unterman and Baumann, 1990).

Differentiation of the genus *Buchnera* from other genera

Since different evolutionary lineages of nonculturable endosymbionts are found within aphids, carpenter ants, and tsetse flies, their initial putative identification is at the level of the insect host (Fig. BXII.γ.196). In *Wigglesworthia* (endosymbionts of tsetse flies), 16S rDNA is upstream of 23S rRNA and this is a useful differential character (Aksoy, 1995a, b). In the carpenter ant endosymbionts the 16S rDNA is not upstream of 23S rDNA, and it appears that the rRNA genes are organized into at least two transcription units (C. Elsishans and R. Gross, personal communication). It is not known if in carpenter ant endosymbionts *aroE* is upstream of the 23S rDNA, as is the case in *Buchnera*. The members of the *Enterobacteriaceae* are mostly free-living, readily identifiable bacteria with their rRNA genes arranged in the order 16S–23S–5S.

Taxonomic Comments

There are over 4000 species of aphids (Blackman and Eastop, 1984), of which only 28 have been characterized by molecular methods. Consequently, our conclusions concerning *Buchnera* are based on a very small sample of aphid species. 16S rDNA has been useful for showing the monophyletic origin of the aphid symbiosis. It appears also to be useful for the differentiation of major aphid subgroups, as for example the *Aphididae* and possibly other families (Fig. BXII.γ.197a). 16S rDNA, however, is far too conserved for the elucidation of relationships between endosymbionts of closely related aphids. We have had some success with the use of a portion of *trpB* for this purpose (Fig. BXII.γ.197b). However, other informational molecules should also be tried.

The name *Buchnera aphidicola* as currently used designates the whole aphid endosymbiont lineage (Fig. BXII.γ.197a). The 16S rDNA sequence difference between *Buchnera* from *S. graminum* and *Buchnera* from *S. chinensis* (Sg and Sc in Fig. BXII.γ.197a) is approximately the same as that between *E. coli* and *Proteus vulgaris*. This clearly indicates that in subsequent studies *Buchnera* should be subdivided into new species. For this purpose, it is necessary to study a molecule that is less conserved than the 16S rDNA, and it is also necessary to know the range of variation within the endosymbionts of a single aphid species. In addition, since there is co-speciation between the host and the endosymbiont, the phylogeny of the host should also be elucidated to serve as an aid in the recognition of new endosymbiont species.

Further Reading

Baumann, P., L. Baumann, C.-Y. Lai, D. Rouhbakhsh, N.A. Moran and M.A. Clark. 1995. Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. Annu. Rev. Microbiol. *49*: 55–94.

Douglas, A.E. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. Annu. Rev. Entomol. *43*: 17–37.

Ishikawa, H. 1989. Biochemical and molecular aspects of endosymbiosis in insects. Int. Rev. Cytol. *116*: 1–45.

Moran, N.A. and A. Telang. 1998. Bacteriocyte-associated symbionts of insects - A variety of insect groups harbor ancient prokaryotic endosymbionts. Bioscience *48*: 295–304.

List of species of the genus Buchnera

1. **Buchnera aphidicola** Munson, Baumann and Kinsey 1991b, 567[VP]
a.ph' di.co.la. M.L. fem. n. *aphidicola* aphid dweller.

The characteristics are those described for the genus. Morphological features are depicted in Figs. BXII.γ.194 and BXII.γ.195.

The mol% G + C of the DNA is: 27 (determined from the nucleotide sequence of over 100 kb of DNA).

Type strain: Endosymbiont of the aphid *Schizaphis graminum*.

GenBank accession number (16S rRNA): M63246.

1. Buffer A contains 0.25 M sucrose, 35 mM Tris-HCl (pH 7.6), 25 mM KCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol (Ishikawa, 1982).

Genus VI. **Budvicia** *Aldová, Hausner, and Gabrhelová* in *Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985, 63*^{VP}

J.J. Farmer III

Bud.vi' ci.a. L. fem. n. *Budvicia* derived from Budvicium, the Latin name of the city České Budějovice where the bacterium was first isolated.

Small, straight, rod-shaped cells, nonsporeforming, nonencapsulated, and Gram negative, conforming to the general definition of the family *Enterobacteriaceae*. Contains the enterobacterial common antigen. **Motile, producing peritrichous flagella when grown at 22°C; less motile at 36°C.** Grows on laboratory media including MacConkey agar. Grows slowly on nutrient agar, forming colonies only about 0.1 mm at 24 h, 36°C; colonies are five times larger when grown at 30°C for 24 h. More active biochemically at 25°C than 36°C. Oxidase negative, reduce nitrate to nitrite. **Positive for: H_2S production, urea hydrolysis, and fermentation of glucose, L-arabinose, L-rhamnose, D-xylose, and D-galactose.**

Negative for indole production, Voges–Proskauer, citrate utilization (Simmons), phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, growth in the presence of cyanide (KCN test), malonate utilization, esculin hydrolysis, gelatin hydrolysis (22°C), lipase (corn oil), DNase, and the fermentation of sucrose, dulcitol, salicin, adonitol, *myo*-inositol, D-sorbitol, raffinose, maltose, trehalose, cellobiose, α-methyl-D-glucoside, erythritol, melibiose, glycerol, and D-mannose.

Utilize 29 of 83 carbon sources tested. **Grow at 4°, 10°, 22°, 32°, and 37°C, but not at 42°C.** Complex growth factor requirement: **absolute requirement for nicotinic acid**; other vitamins and amino acids stimulate growth. Susceptible to colistin, nalidixic acid, sulfadiazine, gentamicin, streptomycin, kanamycin, chloramphenicol, and carbenicillin (disk diffusion method on Mueller-Hinton agar); **resistant to penicillin, ampicillin, and cephalothin**. **Isolated from drinking and environmental water** in Europe, and from fecal specimens in United States. **No evidence that it is a pathogen for plants or animals.**

The mol% G + C of the DNA is: 46 ± 1 (Bouvet et al., 1985); 51–54 (Aldová et al., 1983).

Type species: **Budvicia aquatica** Aldová, Hausner, and Gabrhelová *in* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985, 63.

Further descriptive information

Guide to the literature Since the genus name was first published in 1983, there have been a few reports in the literature. "HG group" should be included in computer literature searches to retrieve early references. A search from 1983 to May 1999 yielded only seven citations in MEDLINE and 12 citations in BIOSIS. Most are about the organism (Aldová et al., 1983, 1984; Bouvet et al., 1985), comparative taxonomic studies (Bouvet and Grimont, 1987a), or water bacteriology (Hausner et al., 1986; Schubert and Groeger-Söhn, 1998).

Sources Most isolates of *Budvicia* have been from various types of water: wells, rivers and streams, swimming pools, conduits, environmental, and sewage. Hausner et al. (1986) isolated 170 strains of *B. aquatica* from 21,300 water samples, mainly from wells and water mains. One isolate was from the digestive tract of a shrew in Spain (Bouvet et al., 1985).

B. aquatica has also been isolated from human feces in United States (Enteric Reference Laboratory, CDC, unpublished). However, there is no evidence that *B. aquatica* causes diarrhea or infections of the intestinal tract. Apparently, it has not been isolated from extraintestinal human clinical specimens.

Bacteriocin-like agents Strains of *Budvicia* produce bacteriocin-like agents that Šmarda (1987) termed aquaticins.

Isolation, identification, culture preservation, phenotypic characterization Strains of *Budvicia* are not difficult to grow, and are typical *Enterobacteriaceae* in most respects. Strains grow slowly on nutrient agar, forming colonies only about 0.1 mm at 24 h at 36°C, but about five times larger when grown at 30°C for 24 h (Bouvet et al., 1985). Similarly, the biochemical reactions may be different at the two temperatures; and strains may be more active at 25°C. Schubert and Groeger-Söhn (1998) described a method for its detection and quantification in water and sewage samples. See the chapter on the family *Enterobacteriaceae* in this *Manual* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* gives the results at 36°C for *Budvicia* strains in 47 biochemical tests normally used for identification (Farmer, 2003). There are no genus- or species-specific tests or sequences for the identification of *Budvicia*. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the chapter on the family *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 of the chapter on the family *Enterobacteriaceae*. Several computer programs greatly facilitate analyzing the results. Bouvet et al. (1985) point out that strains of *B. aquatica* could be misidentified as H_2S-positive strains of *Yersinia*, *Citrobacter*, or *Salmonella*.

Taxonomic Comments

Discovery, history, nomenclature, and type strains In 1976 the first strain of an unusual H_2S-producing strain of *Enterobacteriaceae* was isolated at the Public Health Laboratory in České Budějovice, Czechoslovakia (Aldová et al., 1983). From 1976 to 1983, 27 additional strains were isolated from water samples in three different areas of Bavaria. In 1983 Aldová et al. described this collection of strains, which had been given the vernacular name "HG group". All the data indicated that this was a new group that was distinct from all of the described genera and species of *Enterobacteriaceae*. Throughout the paper Aldová et al. (1983) used the term "HG group" for the new organism; however, at the end of the paper there was a separate section with the heading "Note", which stated that after the manuscript had been submitted DNA–DNA hybridization data became available from the laboratory of P.A.D. Grimont of the Institute Pasteur confirming that the group was a new genus of *Enterobacteriaceae*. In this note they stated: "The authors accordingly propose for this genus the name *Budvicia* (from the ancient Latin name 'Budvicium' for the city of České Budějovice where the microorganism was first isolated) and for its only species the name *Budvicia aquatica*. The epithet, as is obvious, is derived from the Latin adjective 'aquaticus'."

It is clear from this paragraph that a formal nomenclatural proposal was actually made. However, since no type strain was designated for *B. aquatica*, the names *Budvicia* and *Budvicia aquatica* were not "validly published" (see the *Bacteriological Code*, pages 28–29), and thus are illegitimate names. This situation was

apparently rectified when the names *Budvicia* Aldová, Hausner, Gabrhelová, Schindler, Petráš and Braná 1985a and *Budvicia aquatica* Aldová, Hausner, Gabrhelová, Schindler, Petráš and Braná 1985a gained standing in nomenclature and were validly published in Validation List 17 in the April 1985 issue of the IJSB (see page 223). Footnote f of Validation List 17 states that the type strain of *Budvicia aquatica* is 20186 (CNCTC 350); "Personal communication to the editor of the IJSB". In 1984, Aldová et al. had published a second paper *that* specifically defined this as the type strain. A sentence on page 234 of Aldová et al. (1984) stated: "As the type strain, we propose strain No. 20186 of the National Dysentery Reference Laboratory, which has been deposited at the Czechoslovak National Collection of Type Cultures, Prague under No. M 350."

However, the 1984 paper of Aldová et al. was not listed in the Literature Cited section of Validation List 17. Validation List 17 would have ended a slightly confusing matter; however, in January, 1985 there was a new set of proposals for *Budvicia* and *Budvicia aquatica*. Bouvet et al. published a paper in the *International Journal of Systematic Bacteriology* proposing *Budvicia* and *Budvicia aquatica* as a new genus and species, and their species appeared to be based on a different type strain. This latter paper made a confusing situation even more confusing. In addition, they compounded the confusion by proposing an unconventional name citation (see Nomenclatural problem 3, below). These four conflicting proposals (Aldová et al., 1983, 1984, 1985a; Bouvet et al., 1985) create four nomenclatural problems that require analysis and discussion.

Nomenclatural problem 1 Priority of *Budvicia* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985 over *Budvicia* Aldová, Hausner, Gabrhelová, Schindler, Petráš and Braná 1985a. The first name clearly has priority because it was validly published first (as an article in the *International Journal of Systematic Bacteriology*, January 1985, pages 60–64). The second name was published in the next issue (April 1985) in Validation List 17 (page 223).

Nomenclatural problem 2 Priority of *Budvicia aquatica* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985 whose type strain is 20186HG01 (ATCC 35567) over *Budvicia aquatica* Aldová, Hausner, Gabrhelová, Schindler, Petráš and Braná 1985a whose type strain is 20186 (CNCTC 350). The first name has priority for the same reason given above.

Nomenclatural problem 3 Are *Budvicia aquatica* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985 and *Budvicia aquatica* Aldová, Hausner, Gabrhelová, Schindler, Petráš and Braná 1985a objective or subjective synonyms? The two species would be objective synonyms if they have the same type strain, but would be subjective synonyms if they have different type strains. The type strains were published with different, but similar, strain numbers (20186HG01 vs 20186). Whether these strain numbers are the same or different is not clear from any of the published papers.

Nomenclatural problem 4 Author citations for *Budvicia* and *Budvicia aquatica*. In their original paper in the *International Journal of Systematic Bacteriology* Bouvet et al. (1985) stated: "To recognize the priority of three authors in the delineation of the new genus and species, we propose the following citation of names: *Budvicia* Aldová, Hausner, and Gabrhelová *in* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985, and *Budvicia aquatica* Aldová, Hausner, and Gabrhelová *in* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985."

It seems confusing and a danger to "stability in nomenclature" to have a name citation that disagrees with the authorship of the original paper. The conventional style for author citations as specified in the Bacteriological Code has been followed here, and the reader is referred to the irregular citation proposed by Bouvet et al. (1985). However, in the chapter on *Tatumella*, an

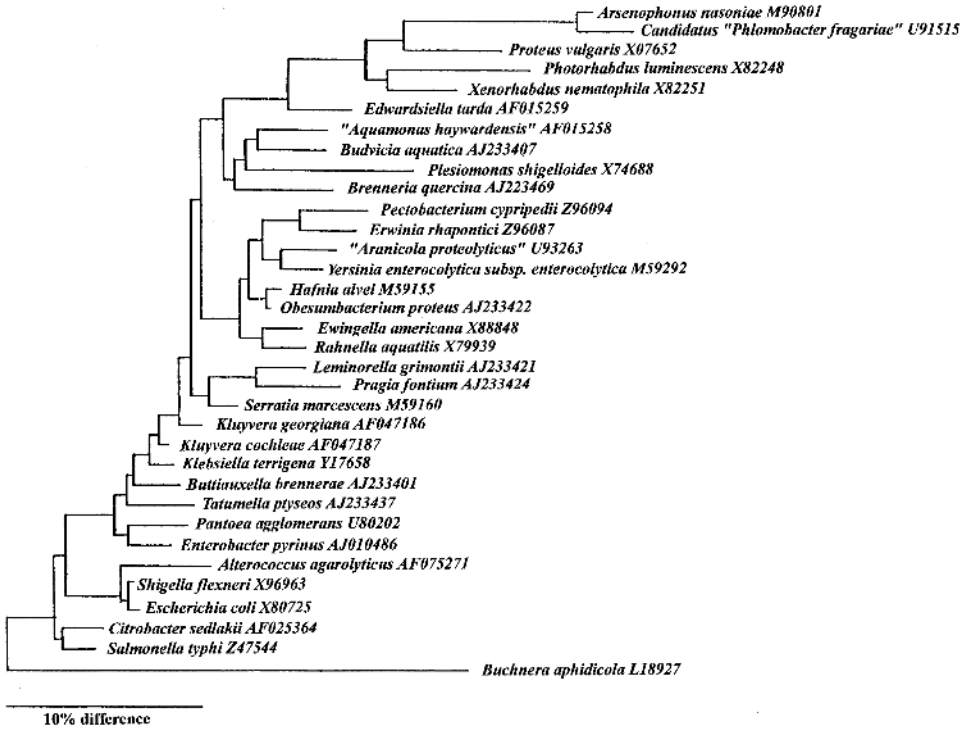


FIGURE BXII.γ.198. Relationship of *Budvicia* and several other new *Enterobacteriaceae* to other organisms in the family based on 16S rDNA sequencing data. Also, see Figure BXII.γ.189 in the family *Enterobacteriaceae*.

irregular name citation was also used. For consistency, the irregular author citation given by Bouvet et al. (1985) was used in this chapter on *Budvicia*.

Interestingly, this is a point that can cause instability in nomenclature. Because an "authors' proposal for an unorthodox name citation" is not specifically covered in the Bacteriological Code, remedial action in the form of a rule modification or Opinion of the Judicial Commission will be needed to guide future writers.

Phylogeny based on DNA–DNA hybridization and 16S rRNA sequencing Based on the DNA–DNA hybridization data *Budvicia* is a distinct and well-defined genus of *Enterobacteriaceae* with its one species *Budvicia aquatica* (Bouvet et al., 1985). None of the other genera of *Enterobacteriaceae* are closely related. Fig.BXII.γ.198 is a tree based on 16S rDNA sequencing that confirms this relationship. The organism on the same branch as *B. aquatica* is a new H_2S producing *Enterobacteriaceae* that will be named *Aquimonas* (originally listed as *Aquamonas* in the GenBank database of sequences). This tree should be compared with the tree published by Spröer et al. (1999), which also shows the distinctness of *Budvicia*. However, this latter tree has *Pragia* and *Leminorella*, two other H_2S-producing genera of *Enterobacteriaceae*, on the same branch.

Schindler et al. (1992) agreed that *Budvicia* strains are distinct from other H_2S-producing *Enterobacteriaceae* based on SDS-PAGE protein patterns.

Further Reading

Aldová, E., O. Hausner, M. Gabrhelová, J. Schindler, P. Petráš and H. Braná. 1983. A hydrogen sulfide-producing gram-negative rod from water. Zentbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. A. *254*: 95–108.

Bouvet, O.M.M., P.A.D. Grimont, C. Richard, E. Aldová, O. Hausner and M. Gabrhelovä. 1985. *Budvicia aquatica*, gen. nov., sp. nov.: a hydrogen sulfide-producing member of the *Enterobacteriaceae*. Int. J. Syst. Bacteriol. *35*: 60–64.

List of species of the genus Budvicia

1. **Budvicia aquatica** Aldová, Hausner, and Gabrhelová *in* Bouvet, Grimont, Richard, Aldová, Hausner and Gabrhelová 1985, 63[VP]

   a.qua'ti.ca. L. fem. adj. *aquatica* living in water; named to show the aquatic habitat of the organism, since all but one of the original strains were isolated from water.

   The characteristics are as previously described for the genus. Biochemical characteristics of the species are given in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Isolated from well water, rivers and streams, swimming pools, water conduits, environmental water, sewage, the digestive tract of a shrew, and from human fecal specimens in United States (Enteric Reference Laboratory, CDC, unpublished). There is no evidence that *B. aquatica* causes diarrhea, infections of the intestinal tract, or extraintestinal infections.

   The mol% G + C of the DNA is: 46 ± 1 (T_m) (Bouvet et al., 1985); 51–54 (T_m and UV spectrophotometry) (Aldová et al., 1983).

   Type strain: 20186HG01, ATCC 35567, Eb 13/82, CDC 0440-84, CNCTC 20186, DSM 5075.

   GenBank accession number (16S rRNA): AJ233407.

   Additional Remarks: The ATCC also lists two other strains ATCC 35566 (Eb 4/81, CNCTC 21930, M 325) and ATCC 51341 (85-01-010).

Genus VII. **Buttiauxella** *Ferragut, Izard, Gavini, Lefebvre and Leclerc 1982, 266[VP] (Effective publication: Ferragut, Izard, Gavini, Lefebvre and Leclerc 1981, 40)*

Peter Kämpfer

But.ti'aux'el.la. M.L. dim. ending *-ella*; M.L. fem. n. *Buttiauxella* named after René Buttiaux, a French microbiologist for his numerous contributions to the taxonomy of *Enterobacteriaceae*.

Straight rods, 0.5–0.7 × 2–3 μm, conforming to the general definition of the family *Enterobacteriaceae*. **Gram negative, motile by peritrichous flagella.** Facultatively anaerobic. Chemoorganotrophic. Psychrotolerant, growing at 4°C. D-Glucose is fermented with the production of acid and gas. Nitrate is reduced to nitrite. Oxidase negative, catalase positive. **Most strains are methyl red positive**. **The majority of strains are indole negative**. **Acids are produced from various carbohydrates, including L-arabinose, cellobiose, maltose, D-mannose, L-rhamnose, D-xylose, and salicin**. 16S rDNA sequence analysis of the type strain of *Buttiauxella agrestis* clearly places the genus in the family *Enterobacteriaceae* within the *Gammaproteobacteria*. Often isolated from fresh water, but also found in soil, and especially in the intestines of snails and slugs from various regions in the world.

The mol% G + C of the DNA is: 47–51.

Type species: **Buttiauxella agrestis** Ferragut, Izard, Gavini, Lefebvre and Leclerc 1982, 266, emend. Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 60 (Effective publication: Ferragut, Izard, Gavini, Lefebvre and Leclerc 1981, 40).

Further descriptive information

Based on 16S rDNA sequence analysis of the type strain, *B. agrestis* was most similar to the type strain of *Klebsiella planticola* (97.9%). Analysis of 16S rDNA of the type strains of the seven species revealed a very high degree of phylogenetic relatedness (99.1–99.7% similarity) (Spröer et al., 1999). The phenotypic description of the genus *Buttiauxella* is largely based on the studies of Gavini et al. (1976b), Ferragut et al. (1981), Farmer (1984b), Farmer et al. (1985a), Brenner (1992a), and Müller et al. (1996). Based on DNA–DNA hybridization it was obvious that the *Buttiauxella* strains comprised seven closely related species, which were very difficult to differentiate phenotypically. The subdivision of the genus *Buttiauxella* is described below (seeTaxonomic Comments).

Cells are straight rods, 0.5–0.7 × 2–3 μm, and can occur singly or in pairs. They are motile at 36°C by peritrichous flagella. *Buttiauxella* strains grow readily on many common media; no growth requirements have been described. A nutrient-rich medium (e.g., Tryptone–Soy agar, sheep-blood agar, or nutrient agar) gives best results. Optimal growth occurs at temperatures ranging from 25°C to 35°C, often yielding better growth than incubation at 37°C. The majority of strains are psychrotolerant, growing (slowly) at 4°C, but not at 41°C.

All members of the genus *Buttiauxella* produce a positive β-galactosidase reaction (ONPG test), although not all produce acid from lactose. The β-glucuronidase test is negative for all

TABLE BXII.γ.210. Characteristics of species of the genus *Buttiauxella*[a,b,c]

Test	Method (s)[d]	*B. agrestis*	*B. brennerae*	*B. ferragutiae*	*B. gaviniae*	*B. izardii*	*B. noackiae*	*B. warmboldiae*
Hybridization group		1	4	2	3	5	6	7
Acetate	Ut.	[+]	d	+	[+]	+	+	+
N-Acetyl-D-galactosamine	Ut.	+	+	+	d	+	+	+
N-Acetyl-L-glutamate	Ut.	+	+	+	+	+	+	+
N-Acetyl-L-glutamine	Ut.	−	−	−	[−]	−	[+]	−
N-Acetylglycine	Ut.	−	−	−	−	−	−	−
Adonitol	Acid., Ut.	−	d	−	d	−	−	−
D-Arabinose	Ut.	d	−	−	d	+	d	+
D-Arabitol	Acid., Ut.	−	d	−	d	−	−	−
L-Arginine dihydrolase	Alcal.	−	d	−	+	[−]	+	+
L-Arginine	Ut.	−	−	−	−	−	[−]	−
Citrate utilization (Simmons)	Ut.,	d	d	−	d	+	+	−
Dulcitol	Acid., Ut.	−	−	−	[−]	−	−	−
L-Fucose	Acid., Ut.	[+]	−	−	d	[+]	−	+
Glycerol	Acid.	d	−	−	−	−	−	−
3-Hydroxybenzoate	Ut.	−	−	−	−	−	−	−
3-Hydroxyphenylacetate	Ut.	−	−	[+]	−	−	−	−
Indole production	Kovacs	−	−	−	d	−	d	−
myo-Inositol	Acid.	−	−	−	−	−	−	+
KCN	Growth	+	+	+	[+]	+	+	−
5-Ketogluconate	Ut.	−	+	−	+	d	+	−
Lactose	Acid.	+	+	−	−	+	−	−
Lactulose	Acid., Ut.	−	−	−	−	−	−	−
L-Lysine decarboxylase	Alcal.	−	−	+	−	−	−	−
Malonate utilization	Ut.	+	+	−	+	+	+	+
Maltitol	Acid., Ut.	[+]	+	+	[+]	d	[+]	−
Melibiose	Acid.	d	+	[+]	−	d	−	−
α-Methyl-D-glucopyranoside	Acid., Ut.	−	d	[−]	−	−	−	−
3-Methyl-D-glucopyranoside	Ut.	+	d	[+]	+	[+]	[+]	−
Methyl red	Acid	+	+	+	+	+	+	+
Mucate	Acid.	d	+	[−]	d	d	+	−
L-Ornithine decarboxylase	Alcal.	+	d	+	−	+	−	−
Palatinose	Acid., Ut.	+	+	+	+	d	+	−
Phenylacetate	Ut.	d	−	d	[−]	d	d	−
L-Phenylalanine deaminase	$FeCl_3$ test	−	−	−	−	−	d	−
3-Phenylpropionate	Ut.	−	−	−	−	−	−	−
L-Proline	Ut.	[+]	+	[+]	+	+	[+]	+
Raffinose	Acid., Ut.	d	+	[−]	−	−	−	−
D-Sorbitol	Acid., Ut.	d	−	+	[−]	−	−	−
Sucrose	Acid.	−	−	−	[−]	−	−	−
D-Tagatose	Acid., Ut.	−	−	−	[−]	−	−	−
Tartrate (Jordan's)	Acid,	d	[−]	−	d	[−]	+	−
Voges–Proskauer	Acetoin	−	−	−	−	−	−	−

[a]Data adapted from Müller et al. (1996).

[b]Symbols: +, positive for 90–100% of strains; [+], positive for 75–89% of strains; d, positive for 25–74% of strains; [−], positive for 11–24% of strains; −, positive for 0–10% of strains. All reactions, unless otherwise stated, were done at 36° ± 1°C and read after 48 h.

[c]All of the strains studied were Gram-negative, oxidase-negative, catalase-positive, D-glucose-fermenting, nitrate-reducing, rod-shaped organisms. All strains grew on MacConkey agar and on Endo agar at 30°C. With few exceptions, all of the strains were positive in standard tests for motility, acid and gas production from D-glucose, fermentation of D-mannitol, salicin, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, D-mannose, galactose, gentiobiose, D-ribose, and arbutin, esculin hydrolysis, and *o*-nitrophenyl-β-D-galactopyranoside (β-galactosidase) test. They were all negative for production of hydrogen sulfide on triple sugar iron, Christensen urease activity, gelatin liquefaction (at 22°C), lipase activity (Tween 80 and corn oil), DNase activity, and production of a yellow pigment, and did not ferment 2-deoxy-D-glucose, 2-deoxy-D-ribose, erythritol, α-D-fucose, D-lyxose, and xylitol.

Almost all of the strains utilized the following compounds as sole carbon and energy sources: *N*-acetyl-D-glucosamine, D-alanine, L-alanine, L-arabinose, arbutin, DL-asparagine, D-cellobiose, D-fructose, D-galactose, D-galacturonate, gentiobiose, D-gluconate, D-glucosamine, D-glucuronate, L-glutamine, L-glutamate, DL-glycerate, glycerol, 4-hydroxybenzoate, 2-ketogluconate, D-lactate, DL-lactate, maltose, maltotriose, mannitol, D-mannose, methyl-α-galactoside, methyl-β-galactoside, methyl-β-D-glucoside, mucate, oxaloacetate, palatinose, protocatechuate, pyruvate, quinate, L-rhamnose, D-ribose, D-saccharate, salicin, L-serine, starch, D-trehalose, and D-xylose.

Almost all of the strains were unable to utilize the following compounds as sole carbon and energy sources: acetamide, acetamidocaprate, *N*-acetyl-DL-methionine, *N*-acetyl-L-proline, *cis*-aconitate, *trans*-aconitate, adipate, β-alanine, allantoin, altrose, DL-2-aminoadipate, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, DL-2-aminobutyrate, DL-3-aminobutyrate, 4-aminobutyrate, DL-2-aminoisobutyrate, DL-3-aminoisobutyrate, 5-aminovalerate, aminoxyacetate, amygdalin, anthranilate, L-arabitol, arabonate, D-arginine, D-asparagine, azelate, benzoate, betaine, 1-butanol, 2-butanol, *n*-butyrate, cadaverine, caprate, caprylate, carni-tine, carnosine, L-citrulline, citraconate, L-cysteinate, dextran, DL-2,4-diaminobutyrate, diaminopimelate, 2,3-diaminopropionate, dimethylglycine, *m*-erythritol, ethanol, ethanolamine, ethylamine, D-fucose, D-glutamate, glutarate, glycinamide, glycogen, glycolate, glycyrrhizinate, 1-hexanol, 1,6-hexandiol, hexylamine, hippurate, histamine, D-histidine, L-histidine, L-homoserine, DL-3-hydroxybutyrate, 4-hydroxybutyrate, DL-2-hydroxycaprate, DL-2-hydroxyisobutyrate, L-2-hydroxyisocaprate, 2-hydroxyisovalerate, DL-δ-hydroxylysine, 2-hydroxyphenylacetate, 4-hydroxyphenylglycine, DL-hydroxyproline, 2-hydroxyvalerate, HQ-β-glucuronide, heptanoate, indole-3-acetate, isobutyrate, isophthalate, itaconate, 2-ketoglutarate, 2-ketoisocaprate, D-leucine, L-leucine, levulinate, D-lysine, D-malate, maleate, D-mandelate, L-mandelate, D-mannoheptulose, D-melezitose, mesaconate, mesoxalate, D-methionine, L-methionine, DL-methioninesulfone, methyl-α-D-glucoside, α-methyl-D-mannoside, β-methyl-D-xyloside, L-norleucine, D-norvaline, L-norvaline, 1,8-octandiol, D-ornithine, phenoxyacetate, D-phenylalanine, phenylglycine, phenyllactate, phosphoenolpyruvate, phthalate, pimelate, poly-D-galactomannan, polygalacturonate, D-proline, propionate, protocatechuate, putrescine, salicylamide, salicylate, sarcosine, sorbate, L-sorbose, spermine, suberate, D-tartrate, *meso*-tartrate, tartronate, taurine, thiamine, tricarballylate, trigonelline, tropate, tryptamine, D-tryptophan, L-tryptophan, L-tyrosine, ureidosuccinate, *n*-valerate, isovalerate, L-valine, xylitol, and L-xylose.

The strains varied in their ability to utilize the following compounds as sole sources of carbon and energy, and these characteristics could not be used to differentiate hybridization groups: D-alanine, L-asparagine, L-aspartate, benzoate, fumarate, D-glucarate, D-glucosaminate, glycerate, glycerophosphate, glycine, glyoxylate, 4-hydroxybenzoate, 4-hydroxyphenylacetate, inulin, DL-isocitrate, DL-isoleucine, 2-ketoglutarate, L-lactate, D-lyxose, L-lyxose, L-malate, L-mannose, phenylpyruvate, D-serine, spermidine, succinate, L-tartrate, L-threonine, and D-turanose.

B. agrestis and *B. izardii* could not be distinguished on the basis of the results of any single test. These groups had to be distinguished on the basis of their overall biochemical profiles. *B. gaviniae* and *B. noackiae* could also not be separated on the basis of the results of a single test, but could be differentiated on the basis of their *N*-acetyl-L-glutamine, adonitol, D-arabitol, L-fucose, mucate, and tartrate reactions. As stated in the text, these two pairs of hybridization groups exhibited the highest levels of interspecies DNA relatedness.

[d]Ut, utilization test; Acid, acidification test; Alcal, alkalinization test.

strains. Hydrogen sulfide formation, Voges–Proskauer, urease, and DNase tests are negative for all strains.

The reduction of nitrous oxide to dinitrogen has been reported in some strains of *B. agrestis* (Kaldorf et al., 1993). Various carbon sources, including carbohydrates, organic acids, and amino acids are utilized as sole sources of carbon (Gavini et al., 1976b; Ferragut et al., 1981; Müller et al., 1996; Table BXII.γ.210).

Isoprenoid quinone Q-8 is the predominant quinone type and a small amount of the menaquinone MK-8 is present in *B. agrestis* ATCC 33320^T (P. Kämpfer, unpublished results). The fatty acid composition as determined by gas chromatographic analysis of 66 strains representing all species (Kämpfer et al., 1997) differed only slightly. All strains contained the fatty acids $C_{14:0}$, $C_{16:0}$, $C_{17:0\ cyclo}$, summed feature ($C_{16:1\ isoI}$ and/or $C_{14:0\ 3OH}$), summed feature ($C_{16:1\ \omega7c}$ and/or $C_{15:0\ iso\ 2OH}$), and summed feature ($C_{18:1\ \omega7c}$, $C_{18:1\ \omega9t}$, and/or $C_{18:1\ \omega12t}$) (Kämpfer et al., 1997), a fatty acid profile typical for the family *Enterobacteriaceae*.

Antimicrobial susceptibility data are summarized by Freney et al. (1988). Members of the genus *Buttiauxella* (13 strains) were usually sensitive to aminoglycosides (gentamicin, tobramicin, and amikacin), doxocycline, and trimethoprim, but resistant to chloramphenicol. Susceptibility to ampicillin, aminoxycillin-clavulanic acid, and tiracillin was observed. Cephalothin and cefoxitin were inactive. *Buttiauxella* produces enterobacterial common antigen (Böttger et al., 1987).

Pathogenicity of *Buttiauxella* for humans and animals has not been documented. Only one *B. agrestis* strain in the study of Müller et al. (1996) was from a human clinical specimen. Farmer et al. (1985a) described seven strains of Enteric Group 59 (now *Buttiauxella noackiae*) originating from sputum and a foot wound. *Buttiauxella* is widely distributed in nature, may be isolated from foods and is occasionally isolated from human sources. Although the natural habitat of *Buttiauxella* was originally thought to be water, the majority of strains have been isolated from the intestines of snails and slugs (Müller et al., 1996).

Enrichment and Isolation Procedures

The cultivation media used for the isolation of *Buttiauxella* are those regarded useful for other members of the family *Enterobacteriaceae*. No specific selective medium has been reported for *Buttiauxella* species. Because of the relatively low clinical significance and the presence of *Buttiauxella* in various habitats, the selective isolation of *Buttiauxella* is rarely required. In most cases, organisms belonging to the genus *Buttiauxella* are isolated with differential media not inhibitory for *Enterobacteriaceae*, such as MacConkey agar, bromothymol blue lactose agar, or phenol red lactose agar. *Buttiauxella* strains grow readily on ordinary nutrient-rich media, and no growth requirements have been described.

Maintenance Procedures

Buttiauxella strains can be maintained in tryptone soy agar stabs or on nutrient agar when kept at room temperature in the dark. They can be preserved by storage in broth containing 10% glycerol or in calf or bovine serum at −80°C. Lyophilization seems to be the best procedure for preservation.

Differentiation of the genus *Buttiauxella* from other genera

No single distinguishing feature is useful for the differentiation of *Buttiauxella* from the other genera of *Enterobacteriaceae* (Table BXII.γ.193 in *Enterobacteriaceae*). Biochemically and based on DNA–DNA hybridization data, *Buttiauxella* are most similar to the genus *Kluyvera*. (Gavini et al., 1983a; Farmer et al., 1985a; Müller et al., 1996); however, the levels of DNA similarity between members of the genera *Buttiauxella* and *Kluyvera* and more than 50 other species belonging to the family *Enterobacteriaceae* were between 15% and 36% (Gavini et al., 1983a; Müller et al., 1996), indicating the separate position of these two genera. In addition, the mol% G + C ratios of *Buttiauxella* and *Kluyvera* are quite different, with values ranging from 47–51% for *Buttiauxella* and 54–58% for *Kluyvera* (Ferragut et al., 1981; Gavini et al., 1983a). *Buttiauxella* and *Kluyvera* exhibit similar fatty acid patterns, but all strains of *K. ascorbata*, *K. cryocrescens*, and *K. georgiana* produced the summed feature $C_{18:1\ \omega7c}$, $C_{18:1\ \omega9c}$, and/or $C_{18:1\ \omega12t}$ in higher amounts (>20% of all fatty acids) than any strain of *Buttiauxella* (Kämpfer et al., 1997). Although *K. georgiana* produced this feature also in amounts >20%, this species can be separated from all *Buttiauxella* species by its high amounts of 15:0 (>8%), which is not found in *Buttiauxella* (Kämpfer et al., 1997). Further physiological and biochemical characters that are helpful for differentiation of the two genera are given in Table BXII.γ.211.

Taxonomic Comments

Based on a numerical taxonomic study of *Enterobacteriaceae*, Gavini et al. (1976b) defined a new group of strains within the family and gave it the vernacular name "group F". Originally these 17 strains isolated from water and unpolluted soils were regarded to be similar to the genus *Citrobacter* because of their negative indole and Voges–Proskauer reactions and their positive citrate and methyl red tests. Subsequently, Ferragut et al. (1981) used DNA–DNA hybridization to compare strains of group F to each other and to named species of *Enterobacteriaceae*. Based on DNA similarity of 82–96% within group F and its low level of DNA similarity to other *Enterobacteriaceae*, they proposed the new genus *Buttiauxella* with one species *Buttiauxella agrestis*. The names *Buttiauxella* and *B. agrestis* were effectively published, but were not validated in the *International Journal of Systematic Bacteriology* before January 1, 1980. They did not appear on the Approved Lists of Bacterial Names (Skerman et al., 1980), but both names have now been validly published (Ferragut et al., 1982) and have standing in nomenclature. Four additional "group F" strains were only 62–66% related to *B. agrestis*. Based on phenotypic differences, three of these strains were placed in a group that was given the name Enteric Group 63, and the remaining strain was designated as Enteric Group 64 (Farmer et al., 1985a). In 1981, another group of strains, phenotypically similar to *Pantoea agglomerans* (formerly *Enterobacter agglomerans*), except for a positive arginine dihydrolase reaction, was reported and named Enteric Group 59 (Farmer et al., 1985a). Eight isolates, originating from sputum (6), a foot wound (1), and ham (1), were studied.

Between 1984 and 1988, Müller et al. (1995a, b, 1996)

TABLE BXII.γ.211. Differentiation of *Buttiauxella* and *Kluyvera*[a,b]

Test	*Buttiauxella*	*Kluyvera*
Mol% G + C content	47–51	54–58
Indole production	−	+[c]
Citrate (Simmons)	d	[+]
Sucrose fermentation	−	[+]
Raffinose fermentation	[−]	+
α-Methyl-D-glucose fermentation	−	+
Melibiose fermentation	d	+
N-Acetyl-L-glutamate utilization	+	−

[a]Data adapted from Ferragut et al. (1981), Gavini et al. (1983a), Müller et al. (1996)

[b]Symbols: +, positive for 90–100% of strains; [+], positive for 75–89% of strains; d, positive for 25–74% of strains; [−], positive for 11–24% of strains; − positive for 0–10% of strains.

[c]Except for *K. cochleae*.

screened the intestinal contents of snails and slugs for the presence of *Enterobacteriaceae* and, in addition to *Rahnella* isolates and the infrequently seen species *Ewingella americana* and *Serratia fonticola*, found many strains belonging to the genera *Buttiauxella* and *Kluyvera*.

Two hundred and nineteen strains belonging to the genera *Buttiauxella* and *Kluyvera* were subjected to an extensive DNA similarity study. The results indicated that the strains belonging to the genus *Buttiauxella* comprised seven closely related hybridization groups. One of these (hybridization group 1) corresponded to *B. agrestis*. Hybridization groups 2 through 7 were described as the new species: *B. ferragutiae* (formerly Enteric Group 63), *B. gaviniae* (formerly Enteric Group 64), *B. brennerae*, *B. izardii*, *B. noackiae* (formerly Enteric Group 59), and *B. warmboldiae* (Müller et al., 1996). The levels of similarity obtained for members of the seven species were generally between 45% and 65%, a good indication that all of these groups belong to a single genus. The hybridization groups that exhibited the highest levels of intergeneric similarity were hybridization groups 1 (*B. agrestis*) and 5 (*B. izardii*) and hybridization groups 3 (*B. gaviniae*) and 6 (*B. noackiae*). The relative binding ratios obtained from DNA–DNA hybridization studies between the *Buttiauxella* species are given in Table BXII.γ.212. The levels of DNA similarity between members of the *Buttiauxella* and *Kluyvera* species were generally between 15% and 30% (Müller et al., 1996).

Acknowledgments

I thank Don J. Brenner for his helpful comments and critical reading of this chapter, Hans E. Müller for interesting discussions and for furnishing strains, and W. Ludwig for critical comments and his experience in 16S rDNA sequence analyses.

Further Reading

Brenner, D.J. 1992. Additional genera of *Enterobacteriaceae*. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes, 2nd Ed., Vol. 3, Springer-Verlag, New York. pp. 2922–2937.

Farmer , J.J. III 1984. Other genera of the family *Enterobacteriaceae*. *In* Krieg and Holt (Editors), Bergey's Manual of Systematic Bacteriology, 1st Ed., Vol. 1, The Williams & Wilkins Co., Baltimore. pp. 506–516.

Ferragut, C., D. Izard, F. Gavini, B. Lefebvre and H. Leclerc. 1981. *Buttiauxella*, a new genus of the family *Enterobacteriaceae*. Zentbl. Bakteriol. Mikrobiol. Hyg. 1 Abt. Orig. C *2*: 33–44.

Gavini, F., B. Lefebvre and H. Leclerc. 1976. Positions taxonomiques d'entérobactéries H_2S-par rapport au genre *Citrobacter*. Ann. Microbiol. (Paris) *127a*: 275–295.

Müller, H.E., D.J. Brenner, G.R. Fanning, P.A.D. Grimont and P. Kämpfer. 1996. Emended description of *Buttiauxella agrestis* with recognition of six new species of *Buttiauxella* and two new species of *Kluyvera*: *Buttiauxella ferragutiae* sp. nov., *Buttiauxella gaviniae* sp. nov., *Buttiauxella brennerae* sp. nov., *Buttiauxella izardii* sp. nov., *Buttiauxella noackiae* sp. nov., *Buttiauxella warmboldiae* sp. nov., *Kluyvera cochleae* sp. nov., and *Kluyvera georgiana* sp. nov. Int. J. Syst. Bacteriol. *46*: 50–63.

List of species of the genus Buttiauxella

1. **Buttiauxella agrestis** Ferragut, Izard, Gavini, Lefebvre and Leclerc 1982, 266[VP], emend. Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 60 (Effective publication: Ferragut, Izard, Gavini, Lefebvre and Leclerc 1981, 40.)
a.gres' tis. L. masc. n. *ager, agri*; adj. *agrestis* living in the fields, so named because all original strains were isolated from unpolluted soils and water.

   B. agrestis was called DNA hybridization group 1 in the study of Müller et al. (1996). All 30 strains studied by Müller et al. (1996) were ≥73% DNA-related in 60°C reactions (range 73–100%). In the original description (Ferragut et al., 1981), which was based on the 17 strains originally described by Gavini et al. (1976b), it was observed that 60% of *B. agrestis* strains were malonate positive. In the study of Müller et al. (1996), 96% of the strains were malonate positive. Biochemical tests that are useful for differentiating *B. agrestis* from other *Buttiauxella* species are given in Table BXII.γ.210. The arginine dihydrolase, fucose, glycerol, lactose, melibiose, ornithine decarboxylase, palatinose, and D-sorbitol tests are helpful. Clinical significance, if any, is unknown. Isolated from mollusks, as well as water, soil, and human materials.

   The mol% G + C of the DNA is: 47–50 (T_m).

   Type strain: ATCC 33320, CDC 1176-81, CUETM 77-167, DSM 4586.

   GenBank accession number (16S rRNA): AJ233400.

2. **Buttiauxella brennerae** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 62[VP]
bren' ner.ae. M.L. fem. gen. n. *brennerae* of Brenner, in honor of Frances W. Hickman-Brenner, an American microbiologist, for her contributions to the study of many genera of the *Enterobacteriaceae*.

   Biochemical characteristics are shown in Table BXII.γ.210. Tests that can be used to differentiate *B. brennerae* from other *Buttiauxella* species are the L-arabinose, arginine dihydrolase, fucose, *myo*-inositol, 5-ketogluconate, lysine decarboxylase, and malonate. Clinical significance, if any, is unknown. Isolated from mollusks.

   The mol% G + C of the DNA is: ~50 (T_m).

   Type strain: ATCC 51605, DSM 9396.

   GenBank accession number (16S rRNA): AJ233401.

3. **Buttiauxella ferragutiae** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 60[VP]
fer' ra.gut.i.ae. M.L. fem. gen. n. *ferragutiae* of Ferragut, in honor of Carmen Ferragut, a French microbiologist, for

TABLE BXII.γ.212. Relative binding ratios of DNAs at 60°C between the species of the genus *Buttiauxella* [a,b]

Test	*B. agrestis*	*B. brennerae*	*B. ferragutiae*	*B. gaviniae*	*B. izardii*	*B. noackiae*	*B. warmboldiae*
B. agrestis	81 (73–100)	53 (34–58)	51 (46–54)	56 (51–64)	63 (57–71)	58 (52–64)	56
B. brennerae		85 (68–100)	50 (34–57)	59 (39–68)	53 (44–60)	55 (38–66)	50 (44–55)
B. ferragutiae			100	50 (44–56)	48 (43–56)	55 (52–58)	49
B. gaviniae				84 (72–100)	52 (49–59)	71 (67–77)	49 (42–54)
B. izardii					88 (66–100)	50 (43–62)	48 (45–58)
B. noackiae						88 (81–100)	48 (46–51)
B. warmboldiae							99 (98–100)

[a]Data adapted from Müller et al. (1996).

[b]Results are expressed as percentages, figures in parentheses indicate the range of similarity.

her contribution to the study of the genus *Buttiauxella*, previously called Enteric Group 63.

Biochemical characteristics are shown in Table BXII.γ.210. Positive lysine decarboxylase and D-sorbitol tests and negative ketogluconate and malonate tests can clearly differentiate this species from all other *Buttiauxella* species. Clinical significance, if any, is unknown.

Isolated from water and soil.

The mol% G + C of the DNA is: 48–50 (T_m).

Type strain: ATCC 51602, CDC 1180-81, CUETM 78-31, DSM 9390.

GenBank accession number (16S rRNA): AJ233402.

4. **Buttiauxella gaviniae** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 62^{VP}
ga.vin′ i.ae. M.L. fem. gen. n. *gaviniae* of Gavini, in honor of Françoise Gavini, a French microbiologist, for her contributions to the study of the genus *Buttiauxella*, previously called Enteric Group 64.

Biochemical characteristics are shown in Table BXII.γ.210. Positive arginine dihydrolase, 5-ketogluconate, and palatinose reactions and negative ornithine decarboxylase and raffinose reactions are useful in differentiating *B. gaviniae* from other *Buttiauxella* species. A combination of reaction results is necessary to differentiate this species from *Buttiauxella noackiae*. Clinical significance, if any, is unknown. All but one strain were isolated from mollusks.

The mol% G + C of the DNA is: ~51 (T_m).

Type strain: ATCC 51604, DSM 9393.

5. **Buttiauxella izardii** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 62^{VP}
iz.ard′ i.i. M.L. masc. gen. n. *izardii* of Izard, in honor of Daniel Izard, a French microbiologist, for his contribution to the study of the genus *Buttiauxella*.

Biochemical characteristics are shown in Table BXII.γ.210. No single biochemical characteristic differentiates *B. izardii* from *B. agrestis*, but a combination of several characteristics differentiates these taxa. Useful differential biochemical tests are the L-arabinose, citrate, fucose, *myo*-inositol, ornithine decarboxylase, and raffinose. Clinical significance, if any, is unknown. Isolated from mollusks.

The mol% G + C of the DNA is: ~50 (T_m).

Type strain: ATCC 51606, DSM 9397.

GenBank accession number (16S rRNA): AJ233404.

6. **Buttiauxella noackiae** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 62^{VP}
no.ack′ i.ae. M.L. fem. gen. n. *noackiae* of Noack, in honor of Katrin Noack, who phenotypically characterized the *Buttiauxella* strains, previously called Enteric Group 59.

Biochemical characteristics of 15 strains isolated from snails are shown in Table BXII.γ.210. These characteristics are almost identical to those described for Enteric Group 59; the only exception is the result of the lactose test. All snail isolates lack a yellow pigment and are lactose negative, but seven of the eight Enteric Group 59 strains isolated from humans and food were shown to be lactose positive (Farmer et al., 1985a). It is possible that there is a correlation between the ability to split and metabolize lactose and the ability to survive in humans. Tests that are useful in differentiating *B. noackiae* from other *Buttiauxella* species are the *N*-acetyl-L-glutamine, L-arginine dihydrolase, melibiose, and L-ornithine decarboxylase. A combination of several characteristics is necessary to differentiate *B. noackiae* from other *Buttiauxella* species.

Isolated from mollusks, human sputum, human wounds, and food. Clinical significance, if any, is unknown.

The mol% G + C of the DNA is: ~50 (T_m).

Type strain: ATCC 51607, DSM 9401.

GenBank accession number (16S rRNA): AJ233405.

7. **Buttiauxella warmboldiae** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 62^{VP}
warm′ bold.i.ae. M.L. fem. gen. *warmboldiae* of Warmbold, in honor of Sabine Warmbold, who isolated most strains of the new *Buttiauxella* species at the Staatliches Medizinaluntersuchungsamt Braunschweig.

Biochemical characteristics are shown in Table BXII.γ.210. *Myo*-inositol is utilized and acid is produced by freshly isolated strains for many months, in contrast to all other species belonging to the genus *Buttiauxella*. However, these characteristics are lost after some years of storage; therefore, they are not constitutive. Other biochemical tests that are useful in differentiating *B. warmboldiae* from other *Buttiauxella* species are the L-arabinose, arginine dihydrolase, citrate, fucose, KCN, malonate, maltitol, ornithine decarboxylase, and palatinose. This species is the species that is the most distant from all other *Buttiauxella* species and has the lowest level of DNA–DNA similarity to other *Buttiauxella* species. Clinical significance, if any, is unknown. Isolated from snails.

The mol% G + C of the DNA is: ~52 (T_m).

Type strain: ATCC 51608, DSM 9404.

GenBank accession number (16S rRNA): AJ233406.

Genus VIII. **Calymmatobacterium*** *Aragão and Vianna 1913, 221*^{AL}

GEORGE H. BROWNELL

Ca.lym.ma.to.bac.te′ ri.um. Gr. n. *calymma* mantle, sheath; Gr. dim. neut. n. *bakterion* a small rod; M.L. neut. n. *Calymmatobacterium* the sheathed rodlet.

Pleomorphic rods, 0.5–1.5 × 1.0–2.0 μm, with rounded ends. Occur singly or in clusters. **The cells exhibit single or bipolar condensation of chromatin. Capsules are present.** Gram negative. Nonmotile. The exudate from infected tissues, when stained by Wright's stain or by Giemsa stain, demonstrates **characteristic intracellular organisms in the cytoplasm of large mononuclear phagocytes** ("Donovan bodies"). Can be cultivated *in vivo* in the yolk sac of embryonated chicken eggs, peripheral blood mononuclear cells, and human epithelial (HEp-2) monolayers, or *in vitro* on special egg yolk-containing media; has not been reproducibly cultivated in bacteriologic media. Optimum growth temperature, 37°C. Pathogenic for humans, causing **Donovanosis (granuloma inguinale).**

**Editorial Note:* The type species of the genus *Calymmatobacterium* has been transferred to *Klebsiella* as *Klebsiella granulomatis* (Aragão and Vianna 1913) Carter, Bowden, Bastian, Myers, Sriprakash and Kemp 1999.

The mol% G + C of the DNA is: not known.

Type species: **Calymmatobacterium granulomatis** Aragão and Vianna 1913, 221.

Further descriptive information

In diseased tissue smears stained by Wright's method, *C. granulomatis* occurs within the cytoplasm of large mononuclear monocytes as blue to purple pleomorphic rods surrounded by pink capsules (Fig. BXII.γ.199). The organism may occasionally be observed free in extracellular spaces. The single or bipolar condensation of chromatin gives rise to characteristic "safety-pin" forms. The ultrastructure of the intracellular organisms has been described by Davis and Collins (1969), Dodson et al. (1973), Kuberski et al. (1980), Chandra et al. (1989), Chandra and Jain (1991), and Kharsany et al. (1997). Electron micrographs reveal encapsulated bacilliforms with characteristic Gram-negative cell walls. The presence of fimbriae-like structures have been reported by some investigators: Kuberski et al. (1980), Chandra et al. (1989), and Chandra and Jain (1991).

Calymmatobacterium granulomatis has been clinically proven to be the causal agent of granuloma inguinale (Dienst et al., 1938). The disease is often referred to as donovanosis because initial lesions have been diagnosed in skin areas other than the genital region. Most researchers support the contention that donovanosis is not necessarily a venereal disease but is an infection resulting from intimate contamination and poor hygiene. Infection

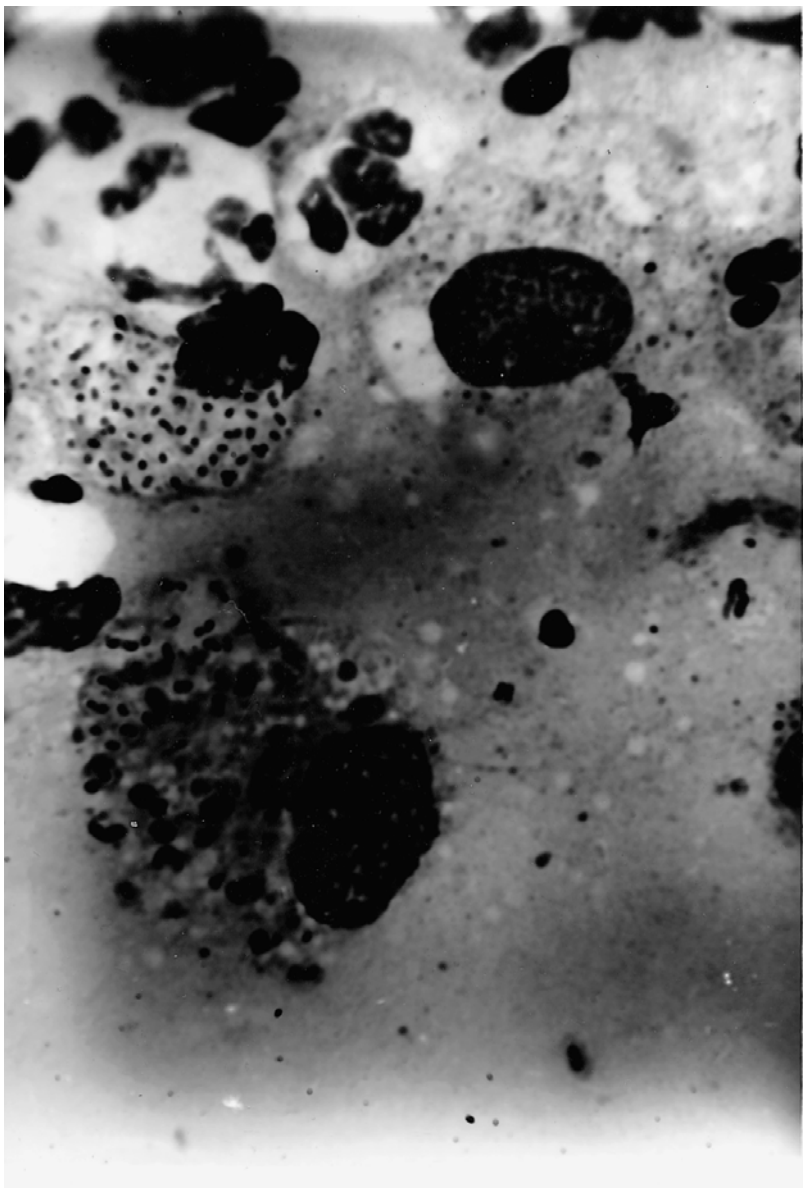


FIGURE BXII.γ.199. Large mononuclear phagocytes filled with *C. granulomatis*. Wright's stain (× 675). (Reproduced with permission from R.B. Dienst and G.H. Brownell in M.P. Starr et al. (Eds.) *The Prokaryotes: a Handbook on Habitats, Isolation and Identification of Bacteria*. p. 1410, 1981, ©Springer-Verlag, New York.)

is usually seen as a chronic, granulomatous, genital ulcerative disease. Donovanosis is an uncommon disease found in specific geographical regions including northern and central Australia, New Guinea, parts of central and south Africa, southeast India, the Caribbean, and parts of South America. The organism is pathogenic only for humans and infection cannot be produced in laboratory animals.

There are no protective antibodies produced by a patient infected with *C. granulomatis*. Once the infection occurs, the disease persists chronically and may spread through the lymphatics to all tissue unless treated with antibiotics. The patient does produce specific sensitizing antibodies as shown by skin testing (Chen et al., 1949). Antibodies can be detected in the serum of patients by complement-fixation procedures (Anderson et al., 1945b; Dulaney and Packer, 1947; Goldberg et al., 1953). The test antigens used in these techniques have included pus from granulomatous lesions, whole or ruptured *C. granulomatis*, or boiled or extracted egg yolk medium following growth of the organism. Immunological studies first noted a relationship between strains of *C. granulomatis* and *Klebsiella pneumoniae* (Packer and Goldberg, 1950). An indirect immunofluorescence test for granuloma inguinale was reported by Freinkel et al. (1992). The antigen consisted of paraffin-embedded tissue sections from lesions containing Donovan bodies. Unabsorbed sera at dilutions of 1:160 were reported to be 100% sensitive and 98% specific. After absorption with *K. pneumoniae* antigen, sera from proven granuloma inguinale patients remained reactive but at lower titers. Antigens obtained from newly reported culture methods well undoubtedly lead to more effective serological tests. (Carter et al., 1997; Kharsany et al., 1997).

Enrichment and Isolation Procedures

At present, the only sources for *C. granulomatis* are the lesions of donovanosis, although new culture methods may soon provide reference strains. An organism was reportedly isolated from human feces (Goldberg, 1962) that had antigenic similarities to *C. granulomatis*. Early reports of the cultivation of *C. granulomatis* in cell-free media include the yolk of embryonated eggs (Anderson, 1944), fresh yolk medium (Dienst et al., 1948), Locke-yolk Dulaney slants (Dulaney et al., 1948), and egg yolk replaced by lactalbumin hydrolysate (Goldberg, 1959). Reports of the successful culturing of *C. granulomatis* in peripheral blood mononuclear cells (Kharsany et al., 1997) or, more conveniently, human epithelial (HEp-2) cells (Carter et al., 1997), should provide the materials that can provide fundamental information about this species that to date is unknown.

The following procedure is given by Morse (1980). The ulcerative lesions are cleansed with sterile, saline-soaked gauze before obtaining samples, in order to decrease contamination and remove tissue debris. Samples of tissue are removed by scraping or by means of a biopsy punch from beneath the border of the lesion, and small cleansed pieces of tissue are minced into small particles. Inoculation is made into the yolk sacs of 5-day-old embryonated eggs. After incubation for 72 h, the organisms can be detected in the yolk sac fluid.

C. granulomatis can also be isolated and grown *in vitro*. For example, a pure culture was isolated by Dienst et al. (1948) by inoculating fresh egg medium exudate aspirated from a pseudobubo of a patient. This isolate was reported to be subcultured and maintained in the same culture medium, and examination of the subcultures revealed large numbers of encapsulated organisms consistent with the morphology of *C. granulomatis*. Dienst et al. (1948) indicated that several factors were important for isolation and cultivation: (a) maintenance of a low oxidation-reduction potential, (b) the requirement for a growth factor found in egg yolk, and (c) use of semisolid media containing 0.12% agar.

Dulaney slants have also been used for isolation and cultivation of *C. granulomatis* (Dulaney et al., 1948). After inoculation of lesion material onto a Dulaney slant, Locke's fluid is added to cover three-quarters of the slant, and the tubes are then incubated in a vertical position for 48–72 h (Morse, 1980). A semisynthetic medium has been devised by Goldberg (1959) for cultivation of laboratory strains of *C. granulomatis*. In this medium, the requirement for egg yolk is replaced by lactalbumin hydrolysate or by papaic digest of soy meal USP.

Recent efforts to culture *C. granulomatis* from biopsy material using these cell-free growth media suggest that they are unreliable. The recommended procedure is therefore the use of fresh monocytes (Kharsany et al., 1996, 1997) or the technically less demanding HEp-2 cell line. The latter employs standard *Chlamydia* culturing procedure (Carter et al., 1997).

Because diagnosis is still based on the observations of *C. granulomatis* in stained biopsy specimens and because of unreliable culture methods, this organism is a prime candidate for PCR primer targeting identification. Using PCR primers designed to target the *pho*E gene encoding for porin protein among klebsiellae and other enterobacteria, Bastian and Bowden (1996) reported amplification of *pho*E gene fragments from biopsy material showing Donovan bodies. The amplified sequences showed close correlation to those from *K. pneumoniae*, *K. rhinoscleromatis*, and *K. ozaenae*, and considerable divergence from corresponding sequences of other enterobacteria analyzed. Using the *pho*E gene primers in combination with primers for *Klebsiella scr*A (sucrose transport gene), reportedly absent in *C. granulomatis*, Carter et al. (1997) obtained *pho*E-positive and *scr*A-negative amplification from original clinical swab material as well as HEp-2 cell cultured isolates. Specific *C. granulomatis* primers have not yet been reported.

Differentiation of the genus *Calymmatobacterium* from other genera

As of this printing, the examination of diseased tissue smears stained by Wright's blood stain or Giemsa stain remains the simplest procedure for identification for *C. granulomatis*. The characteristic appearance of the intracellular organisms (Fig. BXII.γ.199) is specific for the diagnosis of donovanosis. Species-specific PCR primers as well as immunofluorescence tests should soon be available.

Taxonomic Comments

The coccobacillary microorganisms first observed by Donovan (1905) were frequently referred to as "Donovan bodies" when seen in tissue smears from patients with granulomatis lesions in the inguinal region. The Donovan bodies were later called *Calymmatobacterium granulomatis* by Aragão and Vianna (1913). When Anderson et al. (1945a) first isolated the organisms by yolk sac inoculation, they termed the etiologic agent of granuloma inguinale *"Donovania granulomatis"*; however, the name *"Donovania"* did not have priority over *Calymmatobacterium*.

In the first edition of *Bergey's Manual of Systematic Bacteriology*, the genus was not assigned to any family. It has been suggested that it should be placed in the family *Enterobacteriaceae* (Rake, 1948), and this relationship is supported (based on very limited

PCR-generated fragment analysis with "*Klebsiella*-like" sequences) by Bastian and Bowden (1996). Kharsany et al. (1997) observed no cross-reactivity with sera from patients showing Dovonan bodies and laboratory reference strains of *K. pneumoniae*, *K. oxytoca*, or *Enterobacter aerogenes*. Thus, the taxonomic relationships of *Calymmatobacterium* to other bacterial genera are not yet understood, and in the present edition of the *Manual* it seems desirable not to ally the genus with any established family, although it must be noted that the type strain was recently transferred to the genus *Klebsiella* as *Klebsiella granulomatis* (Carter et al., 1999).

List of species of the genus Calymmatobacterium

1. **Calymmatobacterium granulomatis** Aragão and Vianna 1913, 221[AL]
   gran.u.lo' ma.tis. L. dim. n. *granulum* a small grain; Gr. suff. *-oma* a swelling or tumor; M.L. n. *granuloma* a granuloma; M.L. gen. n. *granulomatis* of a granuloma.

   The characteristics are as described for the genus and as depicted in Fig. BXII.γ.199.
   The mol% G + C of the DNA is: not known.
   Type strain: no strain extant.

Genus IX. **Cedecea** *Grimont, Grimont, Farmer and Asbury 1981a, 325[VP] (Enteric Group 15 Farmer, Grimont, Grimont and Asbury 1980b, 295)*

J.J. Farmer III

Ce.de' ce.a. M.L. fem. n. *Cedecea* formed from the abbreviation CDC. The named was coined by P.A.D. Grimont and F. Grimont for the Centers for Disease Control, Atlanta, Georgia, where the organisms were originally recognized as a new group and named Enteric Group 15.

Rod-shaped cells 0.6–0.7 × 1.3–1.9 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile, with five to nine peritrichous flagella. Grow at 15°, 20°, and 37°C. Facultatively anaerobic. **Catalase positive, strong and rapid.** Oxidase negative. Nonpigmented. Reduce nitrate to nitrite.

Positive for methyl red, Voges–Proskauer, citrate utilization (Simmons), motility at 36°C, growth in the presence of cyanide (KCN test), malonate utilization, D-glucose fermentation, and the fermentation of D-mannitol, salicin, maltose, trehalose, cellobiose, melibiose, D-arabitol, D-mannose, and D-galactose. Many strains produce visible gas during fermentation. Lipase (corn oil, Tween 40, Tween 60, Tween 80, and tributyrin) is positive, but gelatin hydrolysis, DNase, chitinase, polygalacturonase, and amylase are negative. Utilize 24 of 97 carbon sources.

Negative for indole production, H_2S production (TSI), urea hydrolysis, phenylalanine deaminase, lysine decarboxylase and the fermentation of dulcitol, adonitol, *myo*-inositol, D-sorbitol, L-arabinose, L-rhamnose, α-methyl-D-glucoside, erythritol, melibiose, glycerol, and mucate.

Susceptible to nalidixic acid, sulfadiazine, trimethoprim, gentamicin, streptomycin, kanamycin, tobramycin, amikacin, tetracycline, minocycline, chloramphenicol, carbenicillin, and furantoin (disk diffusion method on Mueller-Hinton agar); **resistant to colistin, polymyxin, penicillin, ampicillin, and cephalothin**.

Isolated from human clinical specimens that are normally sterile such as blood, urine, gallbladder, and lung tissue; also isolated from other clinical specimens such as throat, sputum, ulcers, and wounds. **An opportunistic pathogen that occasionally causes extraintestinal human infections**, or colonizes body surfaces. Isolated from water, ticks, and insects. A **rarely isolated genus of a *Enterobacteriaceae***. No 16S rRNA sequences of *Cedecea* strains have been reported.

The mol% G + C of the DNA is: 48–52.

Type species: **Cedecea davisae** Grimont, Grimont, Farmer and Asbury 1981a, 325.

Further descriptive information

Literature Since the genus was described in 1981 there have been a few reports in the literature; 11 reports cataloged in MEDLINE and 27 cataloged in BIOSIS. These have described the genus in human clinical specimens and infections, its isolation from natural sources, and its basic physiology-metabolism.

Infections in humans Most of the original isolates of *Cedecea* in the CDC collection were from human respiratory tract specimens, so it was difficult to assess clinical significance (Farmer et al., 1980b). However, a few isolates were from body sites that are normally sterile. Since the original description in 1981, there have been several case reports that suggest clinical significance. The first case of bacteremia due to a strain of *Cedecea* was caused by *Cedecea neteri* (Farmer et al., 1982) and led to the naming of this organism, which was previously without a scientific name ("*Cedecea* species 4"). Other cases of bacteremia have now been described (Perkins, et al., 1986; Aguilera et al., 1995). Several other cases have been described in which a *Cedecea* strain was isolated from a body site that is normally sterile, or thought to be clinically significant (Bae et al., 1975; Hansen and Glupczynski, 1984; Coudron and Markowitz, 1987; Anon et al., 1993). Infections due to *Cedecea* have usually been in elderly hospital patients with debilitating conditions such as heart disease, diabetes, alcoholism, and renal insufficiency. A few isolates have been from human feces, but there is no evidence that any *Cedecea* species causes diarrhea or intestinal infections.

Possible origin of the *Cedecea* strains in human clinical specimens Originally there was little information on this point. Strains of *Cedecea* have now been isolated from well water, ticks, and insects (Jang and Nishijimi, 1990; Kaaya and Okech, 1990; Pellegrini et al., 1992), which may be natural reservoirs and lead to exposure of humans. Berkowitz and Metchock (1995) described bacteria in the feces of hospitalized children that are resistant to third-generation cephalosporins. Antibiotic usage is common in hospitals and selects for strains that have intrinsic or acquired resistance. Since *Cedecea* strains have high intrinsic resistance to penicillin, ampicillin, and the cephalosporin antibiotic cephalothin, they probably have a selective advantage in the feces of hospitalized patients. The reduction of the normal host flora by antibiotic usage could favor the growth and selection

of *Cedecea* strains, which might then colonize certain body sites, and under the right conditions cause infection.

Additional studies and more case reports are needed to better define the pathogenic potential of the five species in the genus. The clinical significance of a *Cedecea* is probably as an infrequent colonizer or infrequent opportunistic pathogen, particularly in older people who are debilitated. More information is needed on ecology, epidemiology, and how humans are exposed to and acquire the organism.

Bacteriology Strains of *Cedecea* grow well on media normally used in enteric bacteriology. Colonies on nutrient agar are about 1.5 mm (24 h, 37°C). They are typical *Enterobacteriaceae* in most of their properties. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media, and methods for biochemical testing, identification, and antibiotic susceptibility. Also see Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*, which gives the percentage positive for the five *Cedecea* species on 47 biochemical tests done at the Enteric Reference Laboratories at the Centers for Disease Control (CDC) with standard media and methods (Farmer, 1995). These tests and computer analysis have proved useful for identification and for differentiating strains of *Cedecea* from other species in the family. Table BXII.γ.213 lists phenotypic tests that are useful for differentiating and identifying the five species of *Cedecea*. Additional descriptive material, including the results for 97 carbon source utilization tests, can be found in the original description of the genus (Grimont et al., 1981a). Chester and Moskowitz (1987) found that *Cedecea* strains produce catalase, and that the catalase reaction was very strong and rapid and could be useful as a screening test.

Taxonomic Comments

History and discovery *Cedecea* was proposed as a new genus in *Enterobacteriaceae* in 1980 (Farmer et al., 1980b; Grimont et al., 1981a). In 1977 it was first recognized and named "Enteric Group 15" as a diagnostic culture was being studied at the CDC. The laboratory's STRAIN MATCHER computer program indicated a group of over a dozen other cultures that were very similar to the diagnostic culture, and all had been reported "unidentified". The strains were lipase-positive (corn oil) and resistant to the antibiotics colistin and cephalothin. Among *Enterobacteriaceae*, these properties are unique to the genus *Serratia*, but the new group differed from *Serratia* because it was negative for DNase and gelatin hydrolysis. Originally, Enteric Group 15 was thought to be a uniform group of strains that would probably be a new species of *Serratia*, intermediate between the "typical" *Serratia* (DNase positive, lipase positive, gelatinase positive) and *Serratia fonticola*, which is negative for all three of these tests (Farmer et al., 1980b). Strains of Enteric Group 15 were sent to the Grimonts in France (Farmer et al., 1980b; Grimont et al., 1981a), who used phenotypic characterization and DNA–DNA hybridization (SI nuclease method) to characterize them. Fifteen strains of Enteric Group 15 were more closely related (Fig. BXII.γ.200) to each other (32–100%) than to strains of the six named *Serratia* species (6–10%) or to other *Enterobacteriaceae* (1–23%). Originally, Enteric Group 15 was defined as a single group of 17 strains, but further study indicated five different DNA hybridization groups (Fig. BXII.γ.200) that were also phenotypically distinct (Table BXII.γ.213). In the original paper proposing the genus *Cedecea*, the two largest hybridization groups were named *C. davisae* and *C. lapagei*. The name *Cedecea neteri* was later given to DNA hybridization group 4 (Farmer et al., 1982). The original vernacular names "*Cedecea* species 3" and "*Cedecea* species 5" are still being used for the two other DNA hybridization groups until more strains are available, or there is a compelling reason to name them (see Other Organisms, below).

Further Reading

Farmer, J.J., III, N.K. Sheth, J.A. Hudzinski, H.D. Rose and M.F. Asbury. 1982. Bacteremia due to *Cedecea neteri* sp. nov. J. Clin. Microbiol. *16*: 775–778.

Grimont, P.A.D., F. Grimont, J.J. Farmer, III and M.A. Asbury. 1981. *Cedecea davisae*, gen. nov., sp. nov. and *Cedecea lapagei*, sp. nov., new *Enterobacteriaceae* from clinical specimens. Int. J. Syst. Bacteriol. *31*: 317–326.

Perkins, S.R., T.A. Beckett and C.M. Bump. 1986. *Cedecea davisae* bacteremia. J. Clin. Microbiol. *24*: 675–676.

List of species of the genus Cedecea

1. **Cedecea davisae** Grimont, Grimont, Farmer and Asbury 1981a, 325[VP]
   da'vi.sae. M.L. gen. n. *davisae* named to honor Betty Davis, the American bacteriologist of the Enteric Bacteriology Laboratories, Centers for Disease Control and Prevention, Atlanta, Georgia, who made many contributions to the biochemical and serological identification of *Enterobacteriaceae* and *Vibrionaceae*.

TABLE BXII.γ.213. Differentiation of the three named and two unnamed *Cedecea* species

Test or property	*Cedecea davisae*	*Cedecea lapagei*	*Cedecea neteri*	*Cedecea* species 3	*Cedecea* species 5
Ornithine decarboxylase	95[a]	0	0	0	50
Sucrose fermentation	100	0	100	50	100
D-Sorbitol fermentation	0	0	100	0	100
Raffinose fermentation	10	0	0	100	100
D-Xylose fermentation	100	0	100	100	100
Melibiose fermentation	0	0	0	100	100
Malonate utilization	91	100	100	0	0
Thiamin required for growth[b]	100	0	0	0	0
Growth at 5°C[b]	100	0	0	0	0

[a]For the first seven tests each number gives the percentage positive after 2 d incubation at 36°C and is based on the data summarized by Farmer (1999). The vast majority of these positive reactions occur within 24 h. Reactions that become positive after 2 d are not considered.

[b]The results for thiamin requirement and growth at 5°C are from Grimont et al. (1981a).

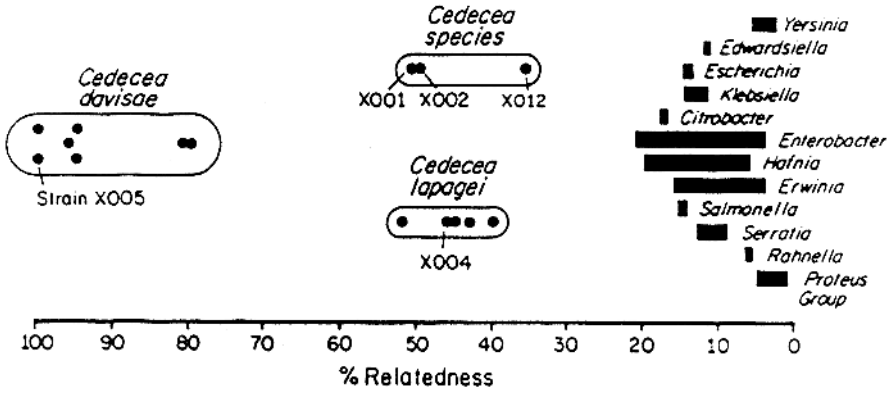


FIGURE BXII.γ.200. Relatedness by DNA–DNA hybridization of the *Cedecea davisae* type stain (X005) to 14 other *Cedecea* strains and to other genera of *Enterobacteriaceae*. Strain X004 is the type strain of *Cedecea lapagei*; X002 is the type strain of *Cedecea neteri*; X001 is the reference strain of "*Cedecea* species 3"; and X012 is the reference strain of "*Cedecea* species 5". (Redrawn with permission from J.J. Farmer III, P.A.D. Grimont, F. Grimont, and M.A. Asbury.)

The characteristics are as given for the genus. All strains require thiamin (0.01 μg/ml) for growth in minimal media and one strain also requires *p*-aminobenzoic acid (Grimont et al., 1981a). Most strains grow at 5°C, unlike the other *Cedecea* species. See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for a more complete phenotypic description based on the results of 47 biochemical tests. One strain produces a strong distinct aroma, the "odor of *Serratia odorifera*", which is apparently due to the pyrazine compound 3-sec-butyl-2-methoxypyrazine (Gallois and Grimont, 1985).

Isolated from human clinical specimens including blood, gallbladder, eye, urine, sputum, throat, wound, feces; probably a rare opportunistic pathogen or colonizer. Perkins et al. (1986) described a case of bacteremia in a 70-year-old woman with a history of heart disease and a diagnosis of bronchitis and chronic obstructive pulmonary disease. Other case reports have described pneumonia in an elderly diabetic with a heart condition (Bae et al., 1975) and a scrotal abscess in a patient with alcoholic liver disease (Bae and Sureka, 1983). Isolated from the cockroaches *Blatta germania* and *Blatta orientalis* (Pellegrini et al., 1992). The 16S rRNA sequence has not been determined.

The mol% G + C of the DNA is: 49–50 (T_m).

Type strain: ATCC 33431, CDC 3278-77, CIP 80.34.

Additional Remarks: The American Type Culture Collection includes four other human strains; two from sputum and one each from gallbladder and wound.

2. **Cedecea lapagei** Grimont, Grimont, Farmer and Asbury 1981a, 325[VP]

la.pa'ge.i. M.L. gen. n. *lapagei* named to honor the late Stephen Lapage, a British bacteriologist who made many contributions to the *Enterobacteriaceae*, bacterial systematics, and particularly as an editor of the Bacteriological Code.

The characteristics are as given for the genus. See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for a more complete phenotypic description based on the results of 47 biochemical tests. All five strains of *C. lapagei* are highly related by DNA–DNA hybridization, but strain 1554-75 had higher divergence values and differed from the other strains in phenotype (Grimont et al., 1981a).

Isolated from human respiratory tract (throat and sputum) and from ticks. Coudron and Markowitz (1987) described an isolate from lung tissue in a case report, but concluded that its etiological role was not proven. Appears to be a tick pathogen. Brun and Texeira (1992a, b) described genital tract infections of the tick species *Boophilus microplus* that resulted in "engorged females". The 16S rRNA sequence has not been determined.

The mol% G + C of the DNA is: 48–52 (T_m).

Type strain: ATCC 33432, CDC 0485-76, CIP 80.35.

Additional Remarks: The American Type Culture Collection includes four other strains, all from human sputum.

3. **Cedecea neteri** Farmer, Sheth, Hudzinski, Rose and Asbury 1983, 438[VP] (Effective publication: Farmer, Sheth, Hudzinski, Rose and Asbury 1982, 777.)

ne'te.ri. M.L. gen. n. *neteri* named to honor Erwin Neter, an American physician and microbiologist who made many contributions to our knowledge of the family *Enterobacteriaceae*, particularly the role of this family in human disease.

The characteristics are as given for the genus. Also see Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for a more complete phenotypic description based on the results of 47 biochemical tests.

Isolated from human blood, sputum, and wound. Farmer et al. (1982) described a case of bacteremia with possible endocarditis in a 62-year-old man with vascular heart disease and recurrent fever and chills of 4 d duration. Anon et al. (1993) described a case of peritonitis after vigorous abdominal surgery in which *Cedecea neteri* was isolated along with *Escherichia vulneris*. Aguilera et al. (1995) described a case of bacteremia in a patient with systemic lupus erythematosus.

The mol% G + C of the DNA is: not reported.

Type strain: ATCC 33855, CDC 0621-75.

Additional Remarks: The American Type Culture Collection includes two other strains, one from human blood (the case report of Farmer et al., 1982) and one from sputum.

Other Organisms

1. "Cedecea species 3"
   The CDC collection has three strains of this organism, from blood, gallbladder, and sputum.
   The mol% G + C of the DNA is: not reported.
   Deposited strain: X001, CDC 4853-73.

2. "Cedecea species 5"
   The CDC collection has only one strain of this organism, from a human toe.
   The mol% G + C of the DNA is: not reported.
   Deposited strain: X012, CDC 3699-73.

The CDC collection has at least 11 other cultures from a variety of human clinical specimens that were reported "*Cedecea* species" because they had high computer identification scores as "genus *Cedecea*" and had characteristics typical for the genus. However, the phenotypic properties of these strains differed from the five *Cedecea* species described above. The data on the two unnamed *Cedecea* species and these 11 "*Cedecea* species" clearly indicate that there are additional species of *Cedecea*. Future studies are needed to better define this collection. There have been two case reports describing *Cedecea* strains that are phenotypically different from the five named species. Hansen and Glupczynski (1984) described a *Cedecea* isolate from a cutaneous ulcer with a purulent discharge in a 79-year-old diabetic with arthritis of both lower limbs. Cultures were positive on days 2, 5, and 43. The isolate was most like *Cedecea davisae* except that it was positive for acetate utilization and D-sorbitol fermentation. The isolate was lost before it could be confirmed by a reference laboratory. Magnum and Radisch (1982) described a *Cedecea* isolate from the postmortem heart blood of a 76-year-old alcoholic man with decubiti, anemia, metabolic acidosis, renal insufficiency, and urinary incontinence. This strain was most like *Cedecea* species 3 except it was Voges–Proskauer positive, fermented sucrose, and grew on acetate agar.

Two additional *Cedecea* species have been described, but they were given the vernacular names of "*Cedecea* species 3" and "*Cedecea* species 5" rather than scientific names (Farmer et al., 1980b; Grimont et al., 1981a). Thus, the two organisms do not have standing in nomenclature, but appear to be unnamed species of *Cedecea* based on DNA–DNA hybridization and phenotypic differences (Table BXII.γ.213). See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for a more complete phenotypic description of this organism based on the results of 47 biochemical tests.

Genus X. **Citrobacter** *Werkman and Gillen 1932, 173*[AL]

WILHELM FREDERIKSEN

Cit.ro.bac' ter. L. n. *citrus* lemon; M.L. n. *bacter* a small rod; M.L. masc. n. *Citrobacter* a citrate-utilizing rod.

Straight rods, ~1.0 μm × 2.0–6.0 μm. Occur singly and in pairs. Conform to the general definition of the family *Enterobacteriaceae*. Usually not encapsulated. Gram negative. Usually **motile by peritrichous flagella**. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow readily on ordinary media. Colonies on nutrient agar are generally 2–4 mm in diameter, smooth, low convex, moist, translucent or opaque, and gray with a shiny surface and entire edge. Mucoid or rough forms may occur occasionally. Oxidase negative. Catalase positive. Chemoorganotrophic. **Citrate can be utilized as a sole carbon source by most strains. Lysine is not decarboxylated.** Alginate and pectate are not decomposed. D-glucose is fermented with the production of acid and gas. The methyl red test is positive; the Voges–Proskauer test is negative. Occur in the feces of humans and some animals; probably normal intestinal inhabitants. Sometimes pathogenic and often isolated from clinical specimens as opportunistic pathogens. Can also be found in soil, water, sewage, and food.

The mol% G + C of the DNA is: 50–52 (T_m).

Type species: **Citrobacter freundii** (Braak 1928) Werkman and Gillen 1932, 173 (*Bacterium freundii* Braak 1928, 140.)

FURTHER DESCRIPTIVE INFORMATION

Members of *Citrobacter* may or may not ferment lactose promptly but nearly always produce β-galactosidase. L-arabinose, maltose, L-rhamnose, trehalose, D-xylose, D-mannitol, and D-sorbitol are fermented rapidly by the majority of strains. Erythritol and *myo*-inositol are rarely attacked.

Ornithine is decarboxylated by almost all strains of *C. koseri*, *C. amalonaticus*, *C. farmeri*, *C. braakii*, *C. sedlakii*, and *C. rodentium*, but not by *C. freundii*, *C. youngae*, and *C. werkmanii*. *C. koseri* and *C. rodentium* do not grow in media containing potassium cyanide (KCN) in contrast to the other species.

Strains of *C. koseri* ferment D-adonitol and D-arabitol, but strains of the other species do not. Malonate is utilized as a sole carbon source by most strains of *C. koseri*, *C. werkmanii*, *C. sedlakii*, and *C. rodentium*, but can be used by less than 15% of the strains of the other species.

The majority of strains of *C. freundii*, *C. youngae*, *C. braakii*, and *C. werkmanii* produce abundant H_2S in the butt of Kligler iron agar and triple-sugar iron agar.

Indole is produced by *C. koseri*, *C. amalonaticus*, *C. farmeri*, and *C. sedlakii* with few exceptions, but few strains of the other species give a positive indole test.

Based on 16S rRNA gene sequence, the genus *Citrobacter* clusters in the family *Enterobacteriaceae* within the *Gammaproteobacteria*. Its nearest relatives are members of the genera *Salmonella* and *Pantoea*.

DNA–DNA hybridization experiments (Brenner et al., 1993) using the hydroxyapatite method showed intraspecific relatedness almost always above 70%, with 5% or less divergence within related sequences, and interspecific relatedness from ~30% to ~70%. *C. farmeri* was closely related to *C. amalonaticus*; *C. youngae*, *C. braakii*, and *C. werkmanii* were related to each other and to *C. freundii* at the 50–70% level. The other interspecies relations were on a lower level (~30% to ~55%).

Some strains of *C. koseri* produce bacteriocins, and many can be induced to produce bacteriophages (Hamon et al., 1974; Markel et al., 1975).

The antigenic structure was developed around the so-called Bethesda-Ballerup group that was considered close to *Salmonella* (Kauffmann and Møller, 1940; West and Edwards, 1954). After its inclusion in *Citrobacter freundii*, the group formed the basis for the elucidation of the O- and H-antigen structure of this species, and its relation to other taxa, especially within the genus *Salmonella*; see Ewing (1986a) for a detailed description. The Vi antigen of *Salmonella* serovar Typhi (*S. typhi* or *S. enterica*, subsp. *enterica*, serovar Typhi) can be found in certain strains of *C. freundii*.

The antigenic structure of *C. koseri* was studied by several groups (Gross and Rowe, 1974, 1983; Popoff and Richard, 1975; Sourek and Aldová, 1976) resulting in three different O- and H-antigen serotyping systems. Sourek and Aldová (1976) also established an O-antigen scheme for *C. amalonaticus*, which they extended in 1988 (Sourek and Aldová, 1988). Van Oye et al. (1975) found that many strains of *C. amalonaticus* reacted in different *Shigella* sera.

Miki et al. (1996) reexamined the 90 reference strains listed in the scheme for *C. freundii* by West and Edwards (1954). They found that 40 of these strains belonged to *C. youngae* as defined by Brenner et al. (1993), 25 to *C. braakii*, 13 to *C. werkmanii*, and only three to the redefined *C. freundii*. Nine O-group-29 strains formerly allocated to the Ballerup group were all identified as *C. braakii*.

With modern typing systems there seems to be less place for serotyping of citrobacters for epidemiological purposes. Both multilocus enzyme electrophoresis (Woods et al., 1992), ribotyping (Papasian et al., 1996; El Harrif-Heraud et al., 1997), PCR methods (Woods et al., 1992; El Harrif-Heraud et al., 1997), and pulsed field gel electrophoresis (Papasian et al., 1996) have been applied, often to elucidate possible outbreaks of *C. koseri* meningitis/cerebral abscesses.

A 32-kDa outer membrane protein (OMP) was found to be a marker of virulence for *C. koseri* (Kline et al.,1988), and possibly a factor of virulence (Li et al., 1990).

Antibiotic sensitivity Strains of *Citrobacter* are naturally susceptible to sulfonamides, trimethoprim, aminoglycosides, chloramphenicol, tetracycline, nalidixic acid, fluoroquinolones, nitrofurantoin, polymyxins, and fosfomycin. Like other enterobacteria they are resistant to erythromycin and other macrolides, lincosamides, fusidic acid, and vancomycin. Resistance to one or more of the former group of antibiotics may be acquired, depending on circumstances, especially antibiotic usage policy in a broad sense.

Citrobacter strains are usually extremely susceptible to the fluoroquinolones. However, like many other enterobacteria they are prone to develop resistance, although this seems to occur rarely. The majority of strains are susceptible to the aminoglycosides, although less so to streptomycin than to newer aminoglycosides.

Almost all strains of *Citrobacter* produce a β-lactamase. *C. freundii* and *C. koseri* differ in the type of β-lactamase produced, *C. freundii* being resistant to cephalosporins and susceptible to carboxypenicillins, while *C. koseri* behave in the reverse way. Resistance to a broad range of newer β-lactam antibiotics may appear in both species; however, imipenem seems to remain active. See Frederiksen and Søgaard (1992) for a broader review of antibiotics and *Citrobacter*. El Harrif-Heraud et al. (1997) described a nosocomial outbreak where six identical strains of *C. koseri* produced an extended spectrum β-lactamase (ESBL), leaving susceptibility only to imipenem, latamoxef, and some combinations with clavulanic acid. The strains harbored a plasmid mediating a SHV-4 type lactamase.

Members of the genus *Citrobacter* occur not only in feces of humans and animals with no disorder but also in water, sewage, soil, and food. Isolates of *C. freundii* recovered from fish were compared with strains from various other sources by Toranzo et al. (1994), and a possible role as a fish pathogen discussed. Citrobacters are found in clinical bacteriology not only in stools but also in urine, sputum, and specimens from bacteremia, meningitis, otitis media, wounds, abscesses, the throat, and autopsies. Their role often seems to be that of an opportunistic pathogen. Cases of neonatal meningitis caused by *C. koseri* have been reported (Gwynn and George, 1973; Kline, 1988). Khashe and Janda (1996) found that an iron-scavenging mechanism such as induction of high molecular mass proteins (72–83 kDa) could be a virulence factor for *C. koseri*.

Although *C. freundii* was once considered an enteropathogen, it seems rather to be a normal inhabitant of the intestine (Sakazaki et al., 1960), but the role in diarrhea is dubious (Sedlák, 1973, Lipsky et al., 1980). In most instances the organism is probably a normal intestinal inhabitant, but some strains produce enterotoxins like many other enterobacteria, and may then act as intestinal pathogens (Guarino et al., 1987, 1989). Septicemia and other disseminated infections, e.g., osteomyelitis, occur with low frequency (Frederiksen and Søgaard, 1992).

C. rodentium has been found only in rodents; it was shown to be the cause of transmissible murine colonic hyperplasia, a disease of laboratory mice (Schauer and Falkow, 1993; Schauer et al., 1995).

Ecology As intestinal inhabitants of humans and animals, citrobacters are excreted into the environment and found in sewage, water, and soil. Apart from this, nothing is known about the ecological role of *Citrobacter* species in the environment.

Phylogenetic position On the basis of their 16S rDNA sequences, citrobacteria group within the *Gammaproteobacteria*, with *Escherichia*, *Erwinia*, *Salmonella*, and *Serratia* species as their nearest relatives.

Enrichment and Isolation Procedures

The majority of *Citrobacter* strains can grow in liquid enrichment media such as selenite broth and tetrathionate broth and on selective isolation media such as *Salmonella–Shigella* agar, deoxycholate–citrate agar, brilliant green agar, and bismuth sulfite agar. Colonies that ferment lactose slowly often resemble *Salmonella* colonies.

Maintenance Procedures

Stock cultures of *Citrobacter* strains may be maintained at room temperature in a semisolid medium containing 1.0% Bacto-casitone (Difco), 0.3% yeast extract, 0.5% NaCl, and 0.3% agar, pH 7.0. The cultures remain viable for up to a year without subculturing if they are sealed with a rubber stopper or a cork that has been soaked in hot paraffin wax. Strains may be preserved indefinitely by lyophilization. They can also be maintained for years as stab cultures in sealed tubes of meat extract agar.

Differentiation of the genus *Citrobacter* from other genera

The differentiation of *Salmonella* from lactose-negative *Citrobacter* proved difficult ever since the "Bethesda–Ballerup" group was recognized as belonging in the genus *Citrobacter*. The most useful tests to distinguish among these lactose-negative strains are lysine decarboxylase and growth in KCN medium. The greatest difficulties arise with the rare lactose/o-nitrophenyl-β-galactosidase (ONPG) positive *Salmonella* strains, the ONPG-negative *Citrobacter* strains (also rare), and, in addition, when H_2S positive *E. coli* are encountered. Table BXII.γ.214 gives some tests useful for differentiation and correct identification of such strains.

Taxonomic Comments

The genus *Citrobacter* was proposed by Werkman and Gillen (1932) for the citrate-utilizing "coli-aerogenes intermediates". The organisms were since described under several names, and *C. freundii* was called *"Escherichia freundii"* by Yale (1939a). The role of citrobacters as possible pathogens was first noticed by Kauffmann and Møller (1940), who described an organism called *"Salmonella ballerup"* that is presently classified in *C. freundii*. Later this biogroup of organisms was removed from the genus *Salmonella* and was called the Ballerup group (Bruner et al., 1949; Harhoff, 1949). Independently of the Ballerup group of organisms, Edwards et al. (1948) and Moran and Bruner (1949) studied a group of bacteria characterized by Barnes and Cherry (1946) and referred to it as the Bethesda group of bacteria. West and Edwards (1954) found that organisms of both the Bethesda and Ballerup groups were biochemically and serologically indistinguishable and combined the two groups into the Bethesda-Ballerup group. Moreover, West and Edwards (1954) and Møller (1954) called attention to the close biochemical relationship between members of the Bethesda-Ballerup group and strains of *"E. freundii"*. Accordingly, Kauffmann (1954) reclassified the Bethesda-Ballerup group into *"E. freundii"*, and later revived the genus *Citrobacter* for *"E. freundii"* (Kauffmann, 1956).

Frederiksen (1970) described a new species that he named *Citrobacter koseri*. Young et al. (1971) described a new genus, *Levinea*, which contained two species, *L. malonatica* and *L. amalonatica*. Ewing and Davis (1972a) published a paper on *Citrobacter diversus*, using the specific epithet "*diversum*" of Werkman and Gillen (1932) for a group of organisms that they considered to belong in *Citrobacter*. It became apparent that *C. koseri*, *L. malonatica*, and *C. diversus* were probably different names for the same species. DNA–DNA hybridization studies by Crosa et al. (1974) showed that *C. diversus* and *L. malonatica* belonged to one hybridization group and that this group and *C. freundii* and *L. amalonatica* were all related with binding ratios around 50–60%.

TABLE BXII.γ.214. The differentiation of H_2S positive *Citrobacter* from ONPG positive *Salmonella* and H_2S positive *Escherichia coli*[a]

Characteristic	*Citrobacter*	*Salmonella*	*E. coli*
Indole	d	−	+
Citrate	+	+	−
KCN (growth)	+	−	−
Lysine	−	+	+
Ornithine	d	+	d
Sucrose	d	−	d
Cellobiose	d	−	−
ONPG	+	+	+
H_2S (iron agar)	+	+	+

[a]For symbols, see standard definitions.

Thus, they all could be considered to belong in the genus *Citrobacter*, with no need for the genus *Levinea*.

Crosa et al. (1974) suggested moving *L. amalonatica* to *Citrobacter*, and Sakazaki et al. (1976), as a result of numerical taxonomy, suggested placing *L. amalonatica* in *Citrobacter* as a species separate from *C. freundii*. The name *C. amalonaticus* was formally proposed by Brenner and Farmer (1981, 1982). Macierevicz (1966) studied a group of organisms that were H_2S-negative and ornithine decarboxylase-positive and proposed the name "*Padlewskia*", without designating a specific epithet for the organisms of this genus. From the biochemical characteristics described it was probable that "*Padlewskia*" organisms and *C. amalonaticus* were identical. This was confirmed when strains from Macierevicz and from Young were compared by the author (unpublished observations).

Werkman and Gillen (1932) also proposed the species name *"Citrobacter intermedium"* (sic) for four of their strains. Frederiksen (1970) showed that the only extant strain, ATCC 6750, was a typical *C. freundii*. The name *"Citrobacter intermedius"*, therefore, was not included on the Approved Lists of Bacterial Names in 1980, and has no nomenclatural standing.

For a time there were three names—*C. koseri*, *L. malonatica*, and *C. diversus*—for the same species, all of which were on the Lists of Approved names. Frederiksen (1990) requested that the name *C. diversus* (Werkman and Gillen, 1932) be placed on the list of rejected names, because it was incorrectly used by Ewing and Davis (1972a), as the organism they described differed in at least eight characteristics from the organism described by Werkman and Gillen as *"Citrobacter diversum"* (sic), and is thus a nomen dubium. This request was granted by the Judicial Commission (1993). The epithet *malonatica* is a junior synonym and should not be used.

The concept of what should be called *C. freundii* was changed when Brenner et al. (1993) published a study showing that a number of atypical *Citrobacter* strains (considered *C. freundii* or *Citrobacter* species) could be arranged in nine genomospecies apart from *C. koseri* and *C. amalonaticus*. They named five new species: *C. farmeri* for *C. amalonaticus* biovar 1, and *C. youngae*, *C. braakii*, *C. werkmanii*, and *C. sedlakii* for strains that had been considered to be *C. freundii* or atypical *C. freundii*. Only 9 of 66 strains were shown genotypically to belong in *C. freundii*. The remaining 57 strains were allocated to seven genomospecies that could all be differentiated phenotypically. Four of them were named (*C. youngae*, *C. braakii*, *C. werkmanii*, and *C. sedlakii*), whereas three (number 9, 10, and 11) were not named, as they contained only three strains each.

Genomospecies 9, containing strains isolated from rodents, was subsequently named *C. rodentium* by Schauer et al. (1995), who studied three additional strains isolated from mouse intestine that were previously called *C. freundii* biotype 4280 (Barthold et al., 1976).

Janda et al. (1994) examined 235 *Citrobacter* strains. Within what was called a *C. freundii* complex, 37% were found to be *C. freundii*, 24% to be *C. youngae*, 13% *C. braakii*, and 6% *C. werkmanii*.

The recent study by Miki et al. (1996) showed that the test strains from the West and Edwards (1954) scheme for the Bethesda-Ballerup group mainly belonged in *C. youngae* (40 strains), *C. braakii* (25 strains), *C. werkmanii* (13 strains), and genomospecies 10 (six strains). Only three strains could be allocated to *C. freundii* in the new sense. Nine strains of the O-29 group ("Ballerup", in which the Vi antigen may occur) were found to belong in *C. braakii*.

The definition of *C. freundii* has thus changed; the new species and *C. freundii* can be differentiated phenotypically and genotypically, but there are no good "key" tests to characterize them; H_2S-positive strains are found in several species, and so are indole-positive strains. The relative distribution within the *C. freundii* complex evidently depends on how the strains included had been selected: strains sent to reference laboratories, or "nonselected" strains from clinical microbiology laboratories (some of the strains used by Janda et al., 1994). There is evidently a need for a thorough characterization of a large unselected collection of *Citrobacter* strains, both phenotypically and genotypically, in order to evaluate the relative importance of the species of the genus as now defined by Brenner and his co-workers.

Differentiation of the species of the genus *Citrobacter*

The differential characteristics of the species of *Citrobacter* are indicated in Table BXII.γ.215. Table BXII.γ.216 lists other characteristics of the species. See also Tables BXII.γ.193, BXII.γ.194, BXII.γ.195, and BXII.γ.196 in the chapter on *Enterobacteriaceae.*

List of species of the genus Citrobacter

1. **Citrobacter freundii** (Braak 1928) Werkman and Gillen 1932, 173[AL] (*Bacterium freundii* Braak 1928, 140.)
freun' di.i. M.L. gen. n. *freundii* of Freund, named after A. Freund, the bacteriologist who first observed that trimethylene glycol was a product of fermentation.

   The morphology is as given for the genus. Usually motile. Usually not encapsulated, although encapsulated strains may occur.

   The colony morphology is similar to that of *Escherichia coli.*

   Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216. Ewing (1986a) listed only 2.1% of strains as indole positive, but among the nine strains included in the study of Brenner et al. (1993), 38% were positive. Janda et al. (1994) found two indole-positive strains among 60 strains allocated to *C. freundii* sensu Brenner et al. (1993). The urease test is positive for most strains if Christensen's method is used, but usually negative if tested for the preformed enzyme.

   Found in humans and animals including mammals, birds, reptiles, amphibians, and fish. Also found in soil, water, sewage, and food. Often found in clinical specimens such as urine, throat, sputum, blood, and wound swabs as an opportunistic or secondary pathogen.

   The mol% G + C of the DNA is: 50–51 (T_m).

   Type strain: ATCC 8090, DSM 30039, IFO 12681, NCTC 9750.

   GenBank accession number (16S rRNA): AJ233408.

2. **Citrobacter amalonaticus** (Young, Kenton, Hobbs and Moody 1971) Brenner and Farmer 1982, 266[VP] (Effective publication: Brenner and Farmer 1981, 1140) (*Levinea amalonatica* Young, Kenton, Hobbs and Moody 1971, 58.)
a.ma.lo.na' ti.cus. Gr. prefix *a* not; M.L. adj. *malonaticus* pertaining to malonate; M.L. adj. *amalonaticus* not pertaining to malonate (i.e., not able to utilize malonate).

   The morphology is as given for the genus. Motile. Not encapsulated.

   Colonies on nutrient agar are translucent to opaque, resembling those of *E. coli.*

   Physiological and biochemical characteristics are indicated in Tables BXII.γ.215 and BXII.γ.216. Some strains are late gelatin liquefiers.

   Found in the feces of humans and animals and in soil,

TABLE BXII.γ.215. Conventional tests useful in differentiating *Citrobacter* species (adapted from Brenner et al., 1993)[a]

Characteristic	1. *C. freundii*	2. *C. amalonaticus*	3. *C. braakii*	4. *C. farmeri*	5. *C. gillenii*	6. *C. koseri*	7. *C. murliniae*	8. *C. rodentium*	9. *C. sedlakii*	10. *C. werkmanii*	11. *C. youngae*
Indole	d[b]	+	d	+	−	+	+	−	+	−	d
Citrate—Simmons	d	+	d[c]	d[c]	d[c]	+	+	−[c]	d[c]	+	d[c]
H_2S in iron agar	d[b]	d[d]	d	−	d	−	d	−	−	+	d
Ornithine	−	+	+	+	−	+	−	+	+	−	−
KCN growth	d[c]	+	+	+	+	−	+	−	+	+	+
Malonate	d	d[d]	−	−	+	+	−	+	+	+	−
Acid from:											
Sucrose	+	d	−	+	d	d	d	−	−	−	d
Melibiose	+	−	d[c]	+	d	−	d	−	+	−	−
Raffinose	d[c]	−	−	+	−	−	d	−	−	−	−
Dulcitol	d	−	d	−	−	d	+	−	+	−	d
Adonitol	−	−	−	−	−	+	−	−	−	−	−
D-Arabitol	−	−	−	−	−	+	−	−	−	−	−

[a]For symbols see standard definitons.

[b]Previous reports (e.g., Ewing, 1986a), on many strains had indole, − (2.1%); and H_2S, + (93.1%).

[c]Late positive reactions may occur in some tests in addition, that may change − to d and d to +.

[d]Previous reports (e.g., Young et al., 1971) had H_2S, −; malonate, −.

TABLE BXII.γ.216. Carbon source utilization reactions of *Citrobacter* species (% positive reactions at 48 h) (adapted from Brenner et al., 1993)[a]

Carbon source	1. *C. freundii*	2. *C. amalonaticus*	3. *C. braakii*	4. *C. farmeri*	5. *C. gillenii*	6. *C. koseri*	7. *C. murliniae*	8. *C. rodentium*	9. *C. sedlakii*	10. *C. werkmanii*	11. *C. youngae*
cis-Aconitate	88	93	89	57	33	100	67	0	100	100	78
trans-Aconitate	13	0	0	57	0	25	0	0	50	83	0
Adonitol	0	0	0	0	0	100	0	0	0	0	0
D-Alanine	100	100	100	71	67	100	100	100	100	100	100
4-Aminobutyrate	25	0	17	7	0	0	33	0	50	0	22
5-Aminovalerate	38	33	50	7	0	6	67	0	33	100	43
D-Arabitol	0	0	0	0	0	100	0	0	0	0	0
Benzoate	0	73	0	93	0	0	0	0	100	0	0
Caprate	0	0	0	0	0	0	0	0	0	0	0
D-Cellobiose	88	100	94	100	67	94	100	100	100	67	78
m-Coumarate	88	0	100	0	33	0	0	0	67	100	96
Dulcitol	13	0	33	0	0	44	100	0	100	0	87
Esculin	0	0	6	0	0	0	0	0	17	0	0
Ethanolamine	0	33	0	14	0	19	0	0	17	17	9
L-Fucose	100	100	94	100	67	100	100	33	100	100	91
Gentiobiose	88	100	89	93	67	88	100	0	100	67	52
Gentisate	100	100	94	93	0	100	67	67	100	100	0
L-Glutamate	75	100	94	86	0	100	100	67	83	83	83
Glycerol	100	100	100	100	100	100	100	0	100	100	100
3-Hydroxybenzoate	100	100	100	100	0	100	67	100	100	100	0
4-Hydroxybenzoate	0	100	0	100	0	0	0	0	100	0	0
3-Hydroxybutyrate	100	20	44	7	0	6	33	0	100	67	74
myo-Inositol	100	0	6	0	67	100	0	0	100	0	0
2-Ketogluconate	100	100	100	100	100	100	100	0	100	100	100
5-Ketogluconate	100	100	100	100	100	100	100	0	0	50	96
2-Ketoglutarate	13	7	22	7	33	6	0	0	33	33	0
DL-Lactate	100	100	100	100	100	100	100	33	100	100	100
Lactose	88	20	78	50	67	56	33	100	100	17	22
Lactulose	88	0	67	7	67	0	33	0	100	17	0
D-Lyxose	63	0	56	0	0	100	100	0	0	83	9
Malonate	0	0	0	0	0	81	0	67	67	50	0
Maltitol	25	7	44	93	0	100	0	0	0	0	0
D-Melibiose	88	7	94	100	100	0	33	0	100	0	0
1-O-CH_3-α-galactoside	88	0	100	100	67	0	0	0	100	0	0
1-O-CH_3-β-galactoside	100	7	83	14	67	100	100	100	100	100	65
3-O-CH_3-D-glucose	63	87	94	86	0	13	67	0	100	100	0
1-O-CH_3-α-D-glucoside	25	13	39	86	0	94	0	0	0	0	0
1-O-CH_3-β-D-glucoside	100	100	100	100	100	100	100	0	100	100	100
Palatinose	25	13	67	100	0	100	67	0	0	0	4
Phenylacetate	25	0	0	0	0	0	0	0	0	0	0
3-Phenylpropionate	75	0	83	0	0	0	0	0	0	100	96
L-Proline	100	87	100	57	33	100	100	100	100	100	87
Propionate	88	80	72	86	0	88	100	67	83	100	78
Protocatechuate	0	100	0	100	0	0	0	0	100	0	0
Putrescine	50	0	39	0	0	0	0	100	0	67	0
D-Raffinose	75	0	6	100	67	0	33	0	0	0	0
L-Sorbose	100	87	6	100	0	0	100	0	0	83	100
Sucrose	100	0	6	100	33	44	33	0	0	0	9
D-Tagatose	13	0	0	36	0	0	0	0	0	0	4
D-Tartrate	13	0	6	0	0	0	0	0	0	100	0
L-Tartrate	0	33	17	36	0	19	33	100	17	67	22
meso-Tartrate	50	40	72	7	0	0	67	100	67	100	78
Tricarballylate	100	93	89	100	0	0	100	100	83	100	4
D-Turanose	0	0	11	36	0	6	0	0	0	0	0
L-Tyrosine	75	0	72	0	0	88	100	0	0	67	74
Xylitol	0	0	0	0	0	19	0	0	0	0	0

[a]All strains (with few single exceptions) utilized the following carbon sources: *N*-acetyl-D-glucosamine, L-alanine, L-arabinose, L-aspartate, citrate, D-fructose, fumarate, D-galactose, D-galacturonate, D-gluconate, D-glucosamine, D-glucose, D-glucuronate, DL-glycerate, D-malate, L-malate, maltose, maltotriose, D-mannitol, D-mannose, mucate, L-rhamnose, D-ribose, D-saccharate, L-serine, D-sorbitol, succinate D-trehalose, and D-xylose. All strains (with two single exceptions) failed to utilize the following carbon sources within 96 h: L-arabitol, betaine, caprate, caprylate, i-erythritol, glutarate, histamine, L-histidine, HQ-beta-glucuronide, itaconate, D-melezitose, quinate, trigonelline, tryptamine, and tryptophan.

water, and sewage. Also found in a variety of human clinical specimens as an opportunistic pathogen.

The mol% G + C of the DNA is: 51–52 (T_m).

Type strain: ATCC 25405, NCTC 10805.

3. **Citrobacter braakii** Brenner, Grimont, Steigerwalt, Fanning, Ageron, and Riddle 1993, 657[VP]

braak' i.i. M.L. gen. n. *braakii* of Braak; named after Hendrik R. Braak, a Dutch microbiologist.

Morphology and cultural characteristics as given for the genus.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in human stools and isolated from animals.

The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51113, CDC 80-58.
GenBank accession number (16S rRNA): AF025368.

4. **Citrobacter farmeri** Brenner, Grimont, Steigerwalt, Fanning, Ageron, and Riddle 1993, 654[VP]
far′ mer.i. M.L. gen. n. *farmeri* of Farmer; named after J.J. Farmer III, an American bacteriologist.

Morphology and cultural characteristics as given for the genus.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in human stools, urine, wounds, and blood.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51112, CDC 2991-81.
GenBank accession number (16S rRNA): AF025371.

5. **Citrobacter gillenii** Brenner, O'Hara, Grimont, Janda, Falsen, Aldová, Ageron, Schindler, Abbott and Steigerwalt 2000, 423[VP] (Effective publication: Brenner, O'Hara, Grimont, Janda, Falsen, Aldová, Ageron, Schindler, Abbott and Steigerwalt 1999, 2623.)
gil.len′ .ii. N.L. gen. n. *gillenii* of Gillen; named after George Francis Gillen, an American microbiologist.

Morphology and cultural characteristics as given for the genus. Physiological and biochemical characteristics are present in Tables BXII.γ.215 and BXII.γ.216. Found in human stool, human urine, human blood, and environment.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51117, CCUG 30796, CDC 4693–86, CIC 106783.

6. **Citrobacter koseri** Frederiksen 1970, 93[AL]
ko.ser.i. M.L. gen. n. *koseri* of Koser; named after Stewart A. Koser, an American bacteriologist.

The morphology is as given for the genus. Motile. Not encapsulated.

Colonies on nutrient agar are translucent to opaque, resembling those of *E. coli.* Cultures often have a distinct fecal odor.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in the feces of humans and animals and in soil, water, sewage, and food. Also isolated from human clinical specimens such as urine, throat, nose, and sputum and wound swabs. Causes neonatal meningitis, often complicated with cerebral abscesses, and often fatal. Some cases occur in small outbreaks.
The mol% G + C of the DNA is: 51–52 (T_m).
Type strain: ATCC 27028.

7. **Citrobacter murliniae** Brenner, O'Hara, Grimont, Janda, Falsen, Aldová, Ageron, Schindler, Abbott and Steigerwalt 2000, 423[VP] (Effective publication: Brenner, O'Hara, Grimont, Janda, Falsen, Aldová, Ageron, Schindler, Abbott and Steigerwalt 1999, 2623.)
mur.lin′ .i.ae. N.L. gen. n. *murliniae* of Murlin; named after Alma C. McWhorter-Murlin, an American microbiologist.

Morphology and cultural characteristics as given for the genus. Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216. Found in human stool, human wound, human blood, human urine, and food.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51118, CCUG 30797, CDC 2970–59, CIC 104556.

8. **Citrobacter rodentium** Schauer, Zabel, Pedraza, O'Hara, Steigerwalt, and Brenner 1996, 362[VP] (Effective publication: Schauer, Zabel, Pedraza, O'Hara, Steigerwalt, and Brenner 1995, 2067.)
ro.den′ ti.um. L. part. adj. used as a gen. n. *rodentium* of rodents (gnawing animals).

Morphology and cultural characteristics as given for the genus; however, four strains were nonmotile, and two showed motility only after 4 days.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216. Citrate utilization was late or absent.

Found in rodents only; includes strains known to produce transmissible murine colonic hyperplasia, associated with the presence of an *eaeA* gene in the isolate.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51116, CDC 1843-73.
GenBank accession number (16S rRNA): AF025363.

9. **Citrobacter sedlakii** Brenner, Grimont, Steigerwalt, Fanning, Ageron, and Riddle 1993, 657[VP]
sed.lak′ i.i. M.L. gen. n. *sedlakii* of Sedlak; named after Jiri Sedlák, a Czechoslovakian bacteriologist.

Morphology and cultural characteristics as given for the genus.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in human stools, blood, and wounds.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51115, CDC 4696-86.
GenBank accession number (16S rRNA): AF025364.

10. **Citrobacter werkmanii** Brenner, Grimont, Steigerwalt, Fanning, Ageron, and Riddle 1993, 657[VP]
werk′ mani.i. M.L. gen. n. *werkmanii* of Werkman; named after Chester H. Werkman, an American bacteriologist.

Morphology and cultural characteristics as given for the genus.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in human stools and blood, and in soil.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 51114, CDC 876-58.
GenBank accession number (16S rRNA): AF025373.

11. **Citrobacter youngae** Brenner, Grimont, Steigerwalt, Fanning, Ageron, and Riddle 1993, 654[VP]
young′ ae. M.L. gen. n. *youngae* of Young; named after Viola M. Young, an American bacteriologist.

Morphology and cultural characteristics as given for the genus.

Physiological and biochemical characteristics are presented in Tables BXII.γ.215 and BXII.γ.216.

Found in human stools, urine, and wounds, and isolated from animals and food.
The mol% G + C of the DNA is: unknown.
Type strain: ATCC 29935, CDC 460-61.

Genus XI. **Edwardsiella** *Ewing and McWhorter 1965, 37*[AL]

RIICHI SAKAZAKI

Ed.ward.si.el' la. M.L. dim. ending *-ella*; M.L. fem. n. *Edwardsiella* named after the American bacteriologist P.R. Edwards (1901–1966).

Straight rods, ~1.0 × 2.0–3.0 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. **Motile by peritrichous flagella**, but nonmotile strains may occur. Facultatively anaerobic. Growth occurs on ordinary media with small colonies (0.5–1.0 mm in diameter) after 24 h incubation. Optimum temperature, 37°C, except for *E. ictaluri*, which prefers a lower temperature. **Nicotinamide and amino acids are required for growth.** Reduce nitrate to nitrite. Ferment D-glucose with the production of acid and often gas. Also **ferment a few other carbohydrates but are inactive compared to many taxa in the family *Enterobacteriaceae*. Usually resistant to colistin but susceptible to most other antibiotics, including penicillin.** Frequently isolated from river fish and cold-blooded animals and their environment, particularly fresh water. Pathogenic for eels, catfish, and other animals, sometimes causing economic losses; also opportunistic pathogen and possibly rare cause of gastroenteritis for humans. A member of the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 53–59.

Type species: **Edwardsiella tarda** Ewing and McWhorter *in* Ewing, McWhorter, Escobar and Lubin 1965, 37.

FURTHER DESCRIPTIVE INFORMATION

Most of studies on *Edwardsiella* concentrated on *E. tarda*; little information is available on other species. Encapsulated strains possessing significant glycocalixes have not been found in members of *Edwardsiella*, but some strains may produce slime substance (Wong et al., 1989). They are afimbriate. Wong et al. (1989) and Janda et al. (1991b) reported that approximately half of strains of *E. tarda*, but not *E. hoshinae* and *E. ictaluri*, produce afimbrial mannose-resistant hemagglutination (MRHA) against guinea pig erythrocytes. Aoki and Holland (1985), who analyzed outer membrane composition of *E. tarda*, found a large number of prominent protein bands in all strains, with major bands present at 27, 35, and 46 kDa, and a cluster of three or four outer membrane proteins located in each strain at 52–56 kDa. Studies of the lipopolysaccharide of three species of *Edwardsiella* indicated that each species may be divided into different chemoforms (Nomura and Aoki, 1985). Since strains of *E. tarda* require nicotinamide and amino acids including cysteine and methionine (d'Empaire, 1969) for their growth and other species of the genus probably have similar requirements, strains of *Edwardsiella* grow less luxuriantly than other members of the *Enterobacteriaceae* and form smaller colonies on ordinary agar plates within 24 h of incubation at 37°C. In contrast with other two *Edwardsiella* species, the optimum growth temperature of *E. ictaluri* is between 25 and 30°C and growth is very slow on plating media, often requiring 2–3 d incubation at 30°C to form typical colonies 1 mm in diameter. Biochemically, *E. ictaluri* is also the least active species of *Edwardsiella*.

Plasmids ranging from 2–120 MDa are harbored in many strains of *Edwardsiella* (Lobb and Rhoades, 1987; Janda et al., 1991a). The presence of R plasmids that mediate antibiotic resistance was reported in *E. tarda* by Aoki et al. (1977). Lobb and Rhoades (1987) recognized that catfish isolates of *E. ictaluri* always harbored two plasmids of 5.7 and 4.9 kb (pCL1 and pCL2, respectively). They suggested that plasmid function may be metabolically important to the host bacterium, or that these plasmids may code for factors important to the virulence, or both. Moreover, Lobb et al. (1993) found that green knife fish isolates of the organisms were serologically distinct from those of catfish and harbored four plasmids with relative mobilities of 6.0, 5.7, 4.1, and 3.1 kb, of which 5.7- and 4.1-kb plasmids strongly hybridized to probes specific for pCL1 and pCL2. Hamon et al. (1969) demonstrated bacteriocin production and sensitivity within *E. tarda*.

For *E. tarda*, two independent serotyping schemes were reported by Sakazaki (1967) and Edwards and Ewing (1972). Currently, an international serotyping scheme combining the two schemes mentioned above and comprising 61 O groups and 45 H antigens has been established by Tamura et al. (1988). Lobb et al. (1993) suggested the possibility of serotyping of *E. ictaluri* as an epidemiological tool. Other typing schemes such as bacteriocin typing, bacteriophage typing, and biotyping have not been studied for *Edwardsiella*.

Strains of *Edwardsiella* are primarily resistant to colistin and polymyxin B, but are usually susceptible to other antimicrobial agents including penicillin G, ampicillin, carbenicillin, streptomycin, kanamycin, gentamicin, tetracycline, chloramphenicol, cephalosporins, sulfadiazine, and, with few exceptions, nalidixic acid (Muyembe et al., 1973; Reinhardt et al., 1985). However, Waltman et al. (1986) reported more resistance to sulfa compounds, streptomycin, and methicillin by disk diffusion testing. These differences may be linked to the source of strains tested. All three species may show large zones around penicillin-impregnated disks. This is an unusual finding for members of the *Enterobacteriaceae*. Clark et al. (1991b) found that strains of *E. tarda*, most originating from human sources, have uniform susceptibility to 22 antibiotics. Although they produced β-lactamase, all strains showed susceptibility to β-lactamase inhibitors. Members of *Edwardsiella* are apparently more susceptible to 2,4-diamino-6,7-diisopropyl pteridine (vibriostatic agent O/129, Sigma) than other *Enterobacteriaceae* (Chatelain et al., 1979; Grimont et al., 1980).

There have been no definitive studies indicating *E. tarda* as a potential cause of human intestinal diseases, but a number of papers from tropical countries such as Madagascar (Fourquet et al., 1975), Zaire (Makulu et al., 1973), Tahiti (Fourquet et al., 1975), Dominica (de Inchaustegui et al., 1976), Panama (Kourany et al., 1977), Cuba (Rakovsky and Aldová, 1965), Australia (Iveson, 1973), India (Bhat et al., 1967; Sakazaki et al., 1971), Thailand (Bockemühl et al., 1971; Ovartlarnporn et al., 1986), Viet-Nam (Nguyen-Van-Ai et al., 1975), Philippines (Tocal and Mezez, 1968), Malaysia (Gilman et al., 1971), and Singapore (Tan et al., 1977) have reported an etiologic relationship of this species to diarrheal diseases. On the other hand, *E. tarda* is also found in the feces of healthy people, although the rate of the isolation is extremely low. Onogawa et al. (1976) found only one positive culture from 97,704 food handlers and 25 positive cultures from 255,896 schoolchildren. Makulu et al. (1973) found no *E. tarda* cultures among 841 healthy subjects in Zaire. Iveson (1973) suggested that the number of isolations of *E. tarda* depends upon culture methods for stool specimens, the geographic area of the

study, and the season in which the survey is performed. In those people from whose stool specimens of *E. tarda* have been isolated, the percentage of diarrheal patients has varied from 25% (Kourany et al., 1977) to more than 75% (Bockemühl et al., 1971; Makulu et al., 1973). A higher isolation rate has invariably been recognized among patients with diarrhea than among asymptomatic people (Bhat et al., 1967; Gilman et al., 1971; Makulu et al., 1973; Nguyen-Van-Ai et al., 1975). In tropical countries there is a tendency for *E. tarda* to be isolated from diarrheal stools together with well-established enteropathogens such as *Salmonella*, *Shigella*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and intestinal parasites (Bhat et al., 1967; Bockemühl et al., 1971; Sakazaki et al., 1971; Makulu et al., 1973; Kourany et al., 1977). The incidence is rare in industrialized countries, but intestinal infection with *E. tarda* was also reported by King and Adler (1964), Chatty and Gavan (1968), and Desenclos et al. (1990) in the United States, and by Vandepitte et al. (1983) in Belgium.

Although *E. tarda* may be able to cause diarrhea, the organism should not be considered as an "inherent" pathogen such as *Salmonella* and *Shigella*. Marques et al. (1984) demonstrated the invasiveness of *E. tarda* in HeLa cells. Janda et al. (1991a) confirmed this finding with HEp-2 cells. However, invasive strains of *E. tarda* give constantly negative results in the Sereny test, suggesting that they have no KepA-like locus as is found in *Shigella* (Ullah and Arai, 1983b; Marques et al., 1984). Janda and Abbott, (1993a) suggested that HEp-2 invasion by *E. tarda* was a microfilament-dependent process. Furthermore, they suggested the cell-associated hemolysin, which was originally demonstrated by Watson and White (1979), as a second major virulence marker. The hemolysin is not released in sufficient amounts under usual growth conditions. Activity of the hemolysin is enhanced by Ca^{2+} and Mg^{2+} and may play a role in infection through epithelial cell destruction leading to an inflammatory infiltrate in the intestinal mucosa, or it could destroy villus cells or disturb intestinal absorption function resulting in diarrhea. In addition to these, Ullah and Arai (1983a) and Janda et al. (1991b) suggested that mannose-resistant hemagglutinins, serum resistance, a dermatotoxin, and chondroitinase activity may be related to human pathogenicity of *E. tarda*. These associations are independent of serovars or source of strains. Whether intestinal infection with *E. tarda* in humans is linked to strains possessing these virulence factors remains to be determined. Bockemühl et al. (1983) reported that some *E. tarda* strains produced a heat-stable enterotoxin (ST) similar to that produced by enterotoxigenic *Escherichia coli*. However, the enterotoxigenic activity was recognized only in culture supernatants concentrated by ultrafiltration. Janda et al. (1991a) also tested *E. tarda* for ST-like activity, but all strains were negative.

Extraintestinal infections with *E. tarda* are well documented, although these occur only rarely in most industrialized countries. It causes meningitis, endocarditis, bacteremia, osteomyelitis, urinary tract infections, or wound infection (Gonzalez and Ruffolo, 1966; Field et al., 1967; Chatty and Gavan, 1968; Okubadejo and Alausa, 1968; Sonnenwirth and Kallus, 1968; Jordan and Hadley, 1969; Pankey and Seshul, 1969; Bockemühl et al., 1971; Sachs et al., 1974; Koshi and Lalitha, 1976; Le Frock et al., 1976; Clarridge et al., 1980; Rao et al., 1981; Sechter et al., 1983; Maserati et al., 1985; Martinez, 1987; Vohora and Torrijos, 1988; Wilson et al., 1989a; Vartian and Septimus, 1990; Hargreaves and Lucey, 1990; Coutlee et al., 1992; Walton et al., 1993; Zighelboim et al., 1992). In most cases of extraintestinal infection, *E. tarda* has been an opportunist in persons with underlying diseases or in conditions with predisposing factors. Infants and the aged tend to be most susceptible to infections with *E. tarda*. *E. tarda* appears to cause serious infection in patients with preexisting liver disease or in those with altered levels of available iron. In cases of wound infection, patients who tend to develop severe infection often have a recent history of fishing, swimming, or injury caused by marine-related items. In extraintestinal infections with *E. tarda*, cell-associated hemolysin is also suggested as a virulence factor, in which a possible function of this hemolysin could be the acquisition of host iron through lysis of erythrocyte to release hemoglobin stores.

E. tarda has been infrequently isolated from warm-blooded animals such as dogs, pigs, cattle, monkeys, rats, panthers, skunks, seals, sea lions, and birds (Ewing et al., 1965; Wallace et al., 1966; Arambulo et al., 1967; Chamoiseau, 1967; Sakazaki, 1967; Tocal and Mezez, 1968; d'Empaire, 1969; Otis and Behler, 1973; White et al., 1973; Owens et al., 1974; Nguyen-Van-Ai et al., 1975; Kourany et al., 1977; Coles et al., 1978). *E. tarda* was associated with diseases in some of those animals, but its pathogenic role in warm-blooded animals is unknown.

Two ecological groups of hosts, cold-blooded animals and fish, could be considered as reservoirs of *E. tarda*. Since Sakazaki and Murata (1962) first suggested *E. tarda* as a normal intestinal inhabitant of snakes, it has been recognized that a wide range of reptiles and amphibians including snakes, crocodiles, alligators, toads, lizards, frogs, and turtles, are possible natural reservoirs for *E. tarda* (Wallace et al., 1966; d'Empaire, 1969; Jackson et al., 1969; White et al., 1969, 1973; Iveson, 1971; Makulu et al., 1973; Meyer and Bullock, 1973; Otis and Behler, 1973; Sharma et al., 1974; Van Der Waaiji et al., 1974; de Inchaustegui et al., 1976; Roggendorf and Müller, 1976; Bartlett et al., 1977; Kourany et al., 1977; Wyatt et al., 1979; Tan et al., 1978). On the other hand, Van Damme and Vandepitte (1980) reported the isolation of *E. tarda* from various kinds of river fish in Zaire. It is considered that river fish and their environment seem to constitute the natural habitat of *E. tarda* and to be the most possible source of human infection, at least in tropical countries. Vandepitte et al. (1983) described protracted diarrhea in an infant associated with *E. tarda*, which was possibly introduced from a tropical aquarium fish in the home of the patient. Trust and Bartlett (1974) reported the presence of *E. tarda* in water samples from aquariums containing goldfish or tropical ornamental fish. Wyatt et al. (1979) recognized *E. tarda* in freshwater catfish and in their environment. Human wound infection with this organism resulting from swimming or diving accidents was reported by Chatty and Gavan (1968) and Clarridge et al. (1980).

E. tarda can cause outbreaks of "red disease" in pond-cultured eels (Hoshina, 1962; Wakabayashi and Egusa, 1973) or "emphysematous putrifactive disease" in channel catfish (Meyer and Bullock, 1973). Kusuda et al. (1977) reported *E. tarda* as causing an outbreak in cultured sea beam. Amandi et al. (1982) showed that *E. tarda* is often isolated from salmonid fish and other fish species in the Pacific Northwest of the United States, and that it is pathogenic for chinook salmon, steelhead and rainbow trout, as well as channel catfish.

E. hoshinae is associated with animals but only eight isolates were originally reported (Grimont et al., 1980). From their paper, the presently known habitats of *E. hoshinae* include reptiles, birds, and water. Although two human isolates of this species were included in their report, those came from feces of individuals without diarrhea; thus there is no evidence that *E. hoshinae* can cause human disease. Another species of *Edwardsiella*, *E. ic-*

taluri, is the causative agent responsible for acute septicemia of catfish. Since the first publication by Hawke et al. (1981), this disease has become a significant problem in the aquaculture of channel catfish in the southeastern United States (Waltman et al., 1986). Shotts et al. (1986) suggested that pathogenesis of *E. ictaluri* may proceed through gastrointestinal tract infection. Enteric septicemia caused by *E. ictaluri* occurs when the temperature ranges from 22–28°C and progresses rapidly, resulting in significant mortality. The isolation of *E. ictaluri* from nonictalurid fish, such as the tropical danio or green knifefish, was also documented (Waltman et al., 1985). Janda et al. (1991b) reported that *E. ictaluri* lacked the invasiveness and cell-associated hemolysin recognized in *E. tarda*, and the lower frequency of these two activities in *E. hoshinae*.

Enrichment and Isolation Procedures

E. tarda and *E. hoshinae* can grow on plating agar media used in enterobacteriology, such as MacConkey, xylose-lysine-deoxycholate (XLD), Hektoen enteric, deoxycholate-citrate, and Salmonella–Shigella agars. Like *Salmonella*, strains of *E. tarda* produce distinguishable black colonies on XLD and Hektoen enteric agars because of H_2S production. As they require some growth factors, their growth may be slower on those media than other enteric bacteria.

Makulu et al. (1973) and Wyatt et al. (1979) reported that tetrathionate or selenite broths as enrichment media may be successful for the isolation of *E. tarda*. Iveson (1971, 1973), who carried out a comparative study to evaluate enrichment media to isolate *E. tarda* from the cloacal contents of tiger snakes and human feces, showed that strontium chloride B broth[1] was the most satisfactory for isolating *E. tarda*, as well as *Salmonella*. After 24 h of incubation at 37°C or 43°C, plates of deoxycholate citrate agar are streaked from the enrichment culture.

On the other hand, Wyatt et al. (1979) evaluated several media for the isolation of *E. tarda* from catfish and the environmental water and found that the most effective isolation of *E. tarda* was obtained with selective enrichment in double-strength SS broth[2] followed by plating on single-strength SS agar. They pointed out that salmonellae were not isolated with this method. A 1-ml amount of lactose broth pre-enrichment is transferred to the double-strength SS broth. After incubation at 35°C for 24 h, cultures are streaked on an SS agar plate.

Muyembe et al. (1973) pointed out that resistance of *E. tarda* to 10 μg/ml colistin can be used in isolation. On the isolation agar plate containing 10 μg/ml colistin, most enteric bacteria are inhibited, with the exception of *Serratia*, *Proteus*, *Providencia*, *Morganella*, *Cedecea*, and some strains of *Yersinia* that are colistin-resistant. Little information is available on the isolation of *E. ictaluri*. Agar medium containing colistin is also effective for the isolation of *E. ictaluri*. The optimum growth temperature of *E. ictaluri* is between 25 and 30°C and growth is slow, often requiring 2–3 days incubation to form colonies 1 mm in diameter.

Maintenance Procedures

Strains of *Edwardsiella* usually survive well at least for 5 years without subculture when the culture is stabbed into a tube with semisolid medium. The tube is tightly sealed with a rubber stopper or with paraffin-coated corks and kept at room temperature in the dark without transfer. A semisolid agar containing 1% Bacto-casitone (Difco), 0.3% yeast extract, 0.5% NaCl, and 0.3% agar (pH 7.2) is an excellent choice for this purpose. However, important cultures should also be preserved at −70°C. Freeze-drying can also be used for long-term preservation.

Procedures for Testing Special Characters

L-Pyrrolidonyl aminopeptidase test Dissolve 10 mg of L-pyrrolidonyl-β-naphthylamide (Sigma) in 10 ml of 95% ethanol. Dip sterile cotton swabs into the solution and rotate each swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Dry the swabs overnight at 37°C and store at −20°C. For testing, make a heavy suspension (MacFarland no. 1) of the test culture into 0.2–0.3 ml of 0.1 M phosphate buffer (pH 7.4), then dip the reagent swab into the suspension and incubate at 37°C for 10 min. Finally, add a drop of cinnamaldehyde solution (*p*-dimethylaminocinnamaldehyde [Sigma] 0.5 g; glacial acetic acid 3.5 ml; ethylene glycol-1-methylester 6.0 ml; sodium lauryl sulfate 3.5 g; and distilled water 90 ml; store in a screw-capped brown container in a refrigerator). A positive result is shown by a pink or red color.

β-Galactosidase and β-glucuronidase tests Dissolve 20 mg each of 4-methylumbelliferyl-β-D-galactopyranoside and 4-methylumbelliferyl-β-D-glucuronide (Sigma) in a small volume of dimethyl sulfoxide, and bring the volume to 10 ml with 0.1 M phosphate buffer (pH 7.4). Dip sterile cotton swabs into the solution and rotate each swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Dry the swabs overnight at 37°C and store at −20°C. For testing, make a heavy suspension (MacFarland no. 1) of the test culture in 0.2–0.3 ml of 0.1 M phosphate buffer (pH 7.4). Dip the reagent cotton swab in the suspension, and incubate at 37°C for 30 min. A positive reaction is indicated by development of blue fluorescence under long-wave (360 μm) ultraviolet lamp.

Differentiation of the Genus *Edwardsiella* from Other Genera

Edwardsiella is biochemically somewhat similar to *E. coli*, *Salmonella*, *Citrobacter* spp., *Leminorella* spp., *Proteus*, and *Providencia*, but is easily differentiated based on activities of L-pyrrolidonyl aminopeptidase, β-galactosidase, β-glucuronidase, and phenylalanine (or tryptophan) deaminase, indole production, and maltose and D-xylose fermentation. Table BXII.γ.217 presents differential characteristics of *Edwardsiella* and other similar members of the *Enterobacteriaceae*. In addition, susceptibilities to penicillin and colistin and to the vibriostatic agent O/129 are useful for differentiation of *Edwardsiella*. Strains of *Edwardsiella* may have zones of inhibition around a penicillin-G disk and no zone around a colistin disk, whereas many groups of the *Enterobacteriaceae* usually have the opposite patterns. *Edwardsiella* may show zones around a 10-μg disk of O/129.

Taxonomic Comments

Edwardsiella was independently reported by several investigators early in the 1960s. Sakazaki and Murata (1962) and Sakazaki (1967) reported a new group of organisms in the family *Ente-*

1. Strontium chloride B broth (g/l): Bacto-tryptone (Difco), 5.0; NaCl, 8.0; K_2HPO_4, 1.0; and $SrCl_2$, 34.0. Sterilization is done by heating at 100°C for 30 min (final pH, 5.0–5.5).

2. Double-strength SS broth (g/l): SS agar, dehydrated, 120, and glucose 5. The mixture is stirred without heat to dissolve the ingredients except agar. The agar is removed by filtration through a Whatman no. 1 filter. The broth is heated at 100°C for 5 min and dispensed to 10-ml quantities into a tube (18 × 180 mm). The tubes are steamed for 30 min.

TABLE BXII.γ.217. Differential characteristics of the genus *Edwardsiella* and biochemically related genera[a]

Characteristic	*Edwardsiella*	*Citrobacter*	*Escherichia coli*	*Leminorella*	*Proteus* and *Providencia*	*Salmonella*
H_2S production	D	D	−	+	D	+
Indole production	D	D	+	−	D	−
L-Pyrrolidonyl aminopeptidase	−	+	−	−	−	−
β-Galactosidase	−	+	+	−	−	−
β-Glucuronidase	−	−	+	−	−	D
Phenylalanine deaminase	−	−	−	−	+	−
Maltose fermentation	+	+	+	−	D	+
D-Xylose fermentation	−	+	+	+	D	+
Mol% G + C of DNA	53–59	50–52	48–52	52–53	38–41	53–58

[a]Symbols: +, 90–100% of strains are positive; −, 90–100% of strains are negative; D, different reaction given by different species or biogroups.

robacteriaceae that were isolated from snakes in 1959 and suggested a vernacular name "Asakusa group" for this group. King and Adler (1964) described the isolation of a culture of the organism and gave it the name "Bartholomew group". Later, Ewing et al. (1965) suggested the scientific name *Edwardsiella tarda* for a group of organisms that had been known as biotype 1483-59 in their laboratory since 1959, and indicated that these organisms were similar to those of the Asakusa and Bartholomew groups. Although the genus *Edwardsiella* with the single species *E. tarda* was proposed by Ewing et al. (1965), there was some question about this designation based upon only phenotypic characterization. Brenner et al. (1974b) demonstrated that strains of *E. tarda* were highly related by DNA hybridization, regardless of their source. They also reported that *Edwardsiella tarda* was only 8–29% related to other genera in the family *Enterobacteriaceae* and was distinct from other members of the family.

Grimont et al. (1980) described a new biogroup designated biogroup 1 of *E. tarda* that was distinguished from biochemically typical strains by its acid production from sucrose, D-mannitol, and L-arabinose, but was closely related to the latter by DNA hybridization. Walton et al. (1993) reported the isolation of a single strain of sucrose-positive, but D-mannitol- and L-arabinose-negative *E. tarda* mimicking biogroup 1 from a patient with cholelithiasis.

The second species of the genus was recognized by Grimont et al. (1980) among the organisms formerly identified as atypical *E. tarda*, and the name *Edwardsiella hoshinae* was proposed for this species. Hawke et al. (1981) reported the third species, *Edwardsiella ictaluri*, associated with septicemia and death in catfish.

Hoshina (1962) recognized an organism as an etiologic agent of "red disease" in eels and named it *"Paracolobactrum anguillimortiferum"*. Based on the International Code of Bacterial Nomenclature 1953 and 1962, *"Paracolobactrum anguillimortiferum"* of Hoshina was a legitimate name for this species. Sakazaki and Tamura (1975) suggested a new combination *"Edwardsiella anguillimortifera"* instead of *Edwardsiella tarda*, because they considered the name *E. tarda* was a junior synonym of *"Paracolobactrum anguillimortiferum"*. Farmer et al. (1976) pointed out differences of some biochemical reactions in descriptions between Hoshina (1962) and Ewing et al. (1965), and emphasized the legitimacy and validity of *Edwardsiella tarda*. Unfortunately, no type strain of *"Paracolobactrum anguillimortiferum"* was designated by Hoshina, and strains studied by him were no longer available. In this status, *"P. anguillimortiferum"* should be considered as a doubtful name, and *E. tarda* is the name that should be used to avoid unnecessary confusion in the literature.

ACKNOWLEDGMENTS

Riichi Sakazaki, who died in 2002, spent more than forty years at the Nippon Institute of Biological Sciences. His illustrious career focused on the classification and epidemiology of human and fish pathogens in Japan, particularly pathogenic bacteria in the families *Vibrionaceae* and *Enterobacteriaceae*. His contributions to Japanese bacteriology paralleled those of Drs. Edwards and Ewing in the U.S. He discovered and described many species in the genera *Enterobacter*, *Edwardsiella*, and *Vibrio*, and he developed serotyping schemes for *V. cholerae* and *V. parahaemolyticus*, as well as for several important species of *Enterobacteriaceae*. His research was always careful and comprehensive. He was one of the first Japanese scientists to publish in English language journals, thereby making his impressive accomplishments available to the world. He was recognized as one of the world's foremost experts on the genus *Vibrio* and on many genera in *Enterobacteriaceae*. He was a member of the WHO Subcommittees on the Taxonomy of *Vibrionaceae* and on the Taxonomy of *Enterobacteriaceae* for more than thirty years. He authored chapters on these organisms in three editions of *Bergey's Manual*. He was a good friend to the Bergey's Trust, as well as to all who knew him.

DIFFERENTIATION OF THE SPECIES OF THE GENUS *EDWARDSIELLA*

Table BXII.γ.218 presents the differential characteristics of the species and biogroups of *Edwardsiella*. Table BXII.γ.219 shows additional biochemical features of those organisms. In the H_2S production test, *E. tarda* biogroup 1 and *E. hoshinae* give a weakly positive reaction in the butt of Kligler iron agar but are usually negative in the butt portion of triple sugar iron agar in which acid produced by sucrose fermentation interferes to combine hydrogen sulfide with Fe^{2+}.

List of species of the genus Edwardsiella

1. **Edwardsiella tarda** Ewing and McWhorter *in* Ewing, McWhorter, Escobar and Lubin 1965, 37[AL]
tar′da. L. fem. adj. *tarda* slow (intended meaning was "inactive," referring to the fermentation on only a few carbohydrates compared to many other *Enterobacteriaceae*).

The characteristics are as given for the genus and listed in Tables BXII.γ.218 and BXII.γ.219.

Occurs in a wide variety of animals, rarely in the feces of healthy people. It is an opportunistic human pathogen, which may cause wound infection and probably some cases of diarrheal diseases.

The mol% G + C of the DNA is: 55–58 (T_m).

Type strain: ATCC 15947, DSM 30052.

GenBank accession number (16S rRNA): AF053975 and AF015259.

TABLE BXII.γ.218. Differentiation of species of the genus *Edwardsiella*[a]

Characteristic	*E. tarda*[b]	*E. tarda*[c]	*E. hoshinae*	*E. ictaluri*
Indole production	+	+	d	−
H_2S production (Kligler)	+	(+)[d]	(+)[d]	−
Motility	+	+	+	−
Malonate utilization	−	−	+	−
Fermentation of:				
L-Arabinose	−	+	d	−
Sucrose	−	+	+	−
Trehalose	−	−	+	−
D-Mannitol	−	+[e]	+	−

[a]For symbols, see standard definitions.

[b]Results for most strains of *E. tarda*.

[c]Results for *E. tarda* biogroup 1.

[d]Weakly positive but may be negative in the butt of TSI agar.

[e]Some strains may be negative.

TABLE BXII.γ.219. Biochemical characteristics of the species and biogroups of the genus *Edwardsiella* [a]

Characteristic	*E. tarda*[b]	*E. tarda*[c]	*E. hoshinae*	*E. ictaluri*
Indole production	+	+	+	−
Voges–Proskauer	−	−	−	−
Citrate utilization (Simmons)	−	−	−	−
H_2S production (Kligler)	+	(+)[d]	(+)[d]	−
Lysine decarboxylase	+	+	+	+
Arginine dihydrolase	−	−	−	−
Ornithine decarboxylase	+	+	+	d
Phenylalanine deaminase	−	−	−	−
Gelatinase	−	−	−	−
Urease (Christensen)	−	−	−	−
β-Galactosidase	−	−	−	−
β-Glucuronidase	−	−	−	−
L-Pyrrolidonyl aminopeptidase	−	−	−	−
Lipase (Tween 80)	−	−	−	−
Deoxyribonuclease	−	−	−	−
Malonate utilization	−	−	+	−
Esculin hydrolysis	−	−	−	−
Growth in KCN medium	−	−	−	−
Gas from glucose	+	d	d	d
Acid from carbohydrate:				
Maltose	+	+	+	+
L-Arabinose	−	+	d	−
Sucrose	−	+	+	−
D-Mannitol	−	+[e]	+	−
Trehalose	−	−	+	−
Salicin	−	−	d	−
D-Cellobiose, lactose, melibiose, raffinose, L-rhamnose, D-xylose, adonitol, D-arabitol, dulcitol, *i*-inositol, D-sorbitol, α-methyl-D-glucoside	−	−	−	−

[a]For symbols, see standard definitions.

[b]Results for most strains of *E. tarda*.

[c]Results for *E. tarda* biogroup 1.

[d]Weakly positive but may be negative in the butt of TSI agar.

[e]Some strains may be negative.

Additional Remarks: These sequences are not from the type strain.

2. **Edwardsiella hoshinae** Grimont, Grimont, Richard and Sakazaki 1981b, 216[VP] (Effective publication: Grimont, Grimont, Richard and Sakazaki 1980, 349.)
ho.shi' nae. M.L. gen. n. *hoshinae* of Hoshina; named after the late Toshikazu Hoshina, the Japanese bacteriologist who was one of the first to describe an organism that was probably an *Edwardsiella*.

   The characteristics are as described for the genus and indicated in Tables BXII.γ.218 and BXII.γ.219. Hydrogen sulfide is weakly produced in Kligler iron agar but may be negative in TSI agar.

   Most isolates are from animals. There is no evidence that this species causes diarrhea.

   The mol% G + C of the DNA is: 56–57 (T_m).

   Type strain: 2-78, ATCC 33379, CIP 78-56.

3. **Edwardsiella ictaluri** Hawke, McWhorter, Steigerwalt and Brenner 1981, 400[VP]
ic.ta.lu' ri. Ictalurus the genus name for catfish; M.L. fem. adj. *ictaluri* pertaining to catfish.

   The characteristics are as described for the genus and as indicated in Tables BXII.γ.218 and BXII.γ.219.

   E. ictaluri is the most fastidious of the three *Edwardsiella* species. Growth is very slow on plating media. It seems to prefer a lower temperature, although characteristic biochemical reactions are apparent at 36°C (Tables BXII.γ.218 and BXII.γ.219). Biochemically, it is also the least active of the three *Edwardsiella* species.

   The antibiotic susceptibility by disk diffusion is difficult to determine because the strains grow so poorly on Mueller-Hinton agar at 37°C. They must be incubated at 25°C instead.

   Occurs as a pathogen of catfish.

   The mol% G + C of the DNA is: 53 (Bd).

   Type strain: SECFDL, GA 7752, ATCC 33202, CDC 1976-78.

Genus XII. **Enterobacter** *Hormaeche and Edwards 1960b, 72[AL] Nom. Cons. Opin. 28, Jud. Comm. 1963, 38*

PATRICK A.D. GRIMONT AND FRANCINE GRIMONT

En.te.ro.bac' ter. Gr. neut. n. *enteron* intestine; M.L. masc. n. *bacter* equivalent of bacterium, a small rod; M.L. masc. n. *Enterobacter* intestinal small rod.

Straight rods, 0.6–1.0 × 1.2–3.0 μm, conforming to the general definition of the family *Enterobacteriaceae*. **Motile by peritrichous flagella** (generally 4–6). Gram negative. **Facultatively anaerobic.** Growth occurs readily on ordinary media. Glucose is fermented with production of acid and gas (generally CO_2:H_2 = 2:1). Gas is not produced from glucose at 44.5°C. **Most strains give a positive Voges–Proskauer reaction and a negative methyl red test.** An alkaline reaction occurs in Simmons citrate and malonate broth. Nitrate is reduced to nitrite. H_2S is not produced from thiosulfate. Tetrathionate is not reduced. **Corn oil and tributyrin are not hydrolyzed. Gelatin, DNA, and Tween 80 are either not, or very slowly, hydrolyzed.** L-Arabinose, D-cellobiose, D-fructose,

D-galactose, D-galacturonate, gentiobiose, D-gluconate, D-glucosamine, D-glucose, D-glucuronate, 2-ketogluconate, L-malate, D-mannitol, D-mannose, D-trehalose, and D-xylose utilized by all or almost all strains, as sole source of carbon and energy. L-rhamnose utilized by all strains except *Enterobacter asburiae*. L-Arabitol, ethanolamine, itaconate, 3-phenylpropionate, L-sorbose, D-tartrate, tryptamine, and xylitol are not utilized. *meso*-Erythritol, gentisate, glutarate, and tricarballylate not utilized except by some strains of *Enterobacter gergoviae*. D-melezitose not utilized except by some strains of *Enterobacter sakazakii*. Optimum temperature for growth is 30°C. Most clinical strains grow at 37°C; some environmental strains give erratic biochemical reactions at 37°C. Widely distributed in nature; common in man and animals.

The mol% G + C of the DNA is: 52–60 (Bd).

Type species: **Enterobacter cloacae** (Jordan 1890) Hormaeche and Edwards 1960b72. Nom. Cons. Opin. 28, Jud. Comm. 1963, 38 (*Bacillus cloacae* Jordan 1890, 836.)

Further descriptive information

Phylogenetic treatment The phylogenetic approach using sequences of the *rrs* gene (encoding 16S rRNA) does not provide sufficient resolution when closely related enterobacterial species are studied, and branching is often unreliable. However, some sequence comparisons have corroborated DNA–DNA hybridization studies. *Enterobacter aerogenes* is more closely related to *Klebsiella* species than to *E. cloacae*, the type species of the genus *Enterobacter*. Therefore, in this volume, *E. aerogenes* has been treated with the genus *Klebsiella* under the name *K. mobilis*. *Enterobacter agglomerans* and several species of the *E. agglomerans* complex cluster together and away from *E. cloacae*. *E. agglomerans* has been transferred to a new genus, *Pantoea*, and in this volume, the *E. agglomerans* complex is treated with the genus *Pantoea*. *Enterobacter intermedius* (originally named *E. intermedium*; corrected by von Graevenitz (1990)) has a *rrs* sequence identical with that of *Kluyvera cochleae*. Furthermore, DNA–DNA hybridization has shown the type strains of *E. intermedius* and *K. cochleae* to be 99% related with a thermal instability of hybridized molecules of 1.5°C (D.J. Brenner, personal communication). Therefore, *E. intermedius* is not treated in this chapter. (See the section on Other organisms in the chapter on the genus *Kluyvera*.)

The *Enterobacter* species treated in this chapter do not cluster together in an *rrs* sequence comparison. However, after *rpoB* sequence comparison, all species and subgroups in the *E. cloacae* complex composed of *E. cloacae*, *Enterobacter asburiae*, *Enterobacter hormaechei*, and the type strain of *Enterobacter dissolvens*) form a cluster (P.A.D. Grimont, unpublished data). *Enterobacter cancerogenus/Enterobacter taylorae* and *E. agglomerans* DNA group VII (not a member of *Pantoea*) form a cluster near *Leclercia adecarboxylata*. *Enterobacter amnigenus*, *Enterobacter nimipressuralis*, and *Enterobacter kobei* constitute another cluster. *Enterobacter cowanii* clusters with *E. agglomerans* DNA group IX (not a member of *Pantoea*) (unpublished data). More work is needed to establish criteria for delineating genera and ascertaining the phylogenetic position of all *Enterobacter* species.

Nutrition and growth conditions Best results are obtained when *Enterobacter* cultures are incubated at 30°C. At 37°C, yellow pigmentation of *E. sakazakii* may be weak. *E. sakazakii* is able to grow at 44°C.

Media used in the isolation of *Enterobacter* species are similar to those used for other *Enterobacteriaceae*. On nutrient agar, *E. cloacae*, *E. gergoviae*, *E. amnigenus*, and *E. nimipressuralis* form colonies that are round, 2–3 mm in diameter, and slightly iridescent or flat with irregular edges (Richard, 1984). *E. sakazakii* strains forms bright yellow colonies at 25°C or pale yellow colonies at 37°C, 1–3 mm in diameter. These colonies are smooth, mucoid, or dry (Farmer et al., 1980a).

Differential media that are not inhibitory for the *Enterobacteriaceae* are often used. These are bromothymol blue-lactose agar, phenol red-lactose agar, Drigalski lactose agar, eosin methylene blue agar, or MacConkey agar. It should be remembered that some strains in a species known to produce acid from lactose might fail to do so and thus give lactose-negative colonies on differential media.

In Biotype-100 strips (BioMerieux, Craponne, France), or in a minimal medium containing ammonium sulfate as the nitrogen source, the following compounds serve as sole carbon sources for most *Enterobacter* strains: *N*-acetyl-D-glucosamine, D-alanine, L-alanine, L-arabinose, L-aspartate, D-cellobiose, D-fructose, fumarate, D-galactose, D-galacturonate, gentiobiose, D-gluconate, D-glucosamine, D-glucose, D-glucuronate, L-glutamate, glycerol, 2-ketogluconate, DL-lactate, L-malate, D-mannitol, D-mannose, L-proline, D-ribose, L-serine, succinate, D-trehalose, and D-xylose. L-rhamnose is utilized by all strains except for most *E. asburiae* strains.

Most strains cannot utilize the following substrates as sole carbon and energy sources : L-arabitol, ethanolamine, itaconate, 3-phenylpropionate, and L-sorbose. The following substrates are not utilized except by some strains of *E. gergoviae*: *meso*-erythritol, gentisate, glutarate, histamine, 3-hydroxybenzoate, and tricarballylate. D-melezitose is not utilized, except by some strains of *E. sakazakii*.

Metabolism When pyrroloquinoline quinone (PQQ) is added, gluconate is produced from D-glucose in the presence of iodoacetate under aerobic conditions by all *Enterobacter* species by means of a glucose dehydrogenase. Only *E. gergoviae*, *E. sakazakii*, *E. cowanii*, and *E. hormaechei* genomic group (*E. cloacae* group III) can produce gluconate without added PQQ (Bouvet et al., 1989). Data are not available for *E. kobei* and *E. pyrinus*. A reducing compound, 2-ketogluconate, is also produced from gluconate by *E. gergoviae* by means of a gluconate dehydrogenase (Bouvet et al., 1989). 2,5-Diketogluconate is not produced from 2-ketogluconate (Bouvet et al., 1989).

Most *Enterobacter* species and biogroups give a positive Voges–Proskauer reaction (except *E. kobei*) and Simmons' citrate tests, motility (except some strains of *E. asburiae*); acid production from D-glucose, D-mannitol, salicin, L-arabinose, L-rhamnose (except most strains of *E. asburiae*), D-xylose, trehalose, D-cellobiose, and maltose; hydrolysis of *o*-nitrophenyl-β-D-galactoside (ONPG), and nitrate reduction. All *Enterobacter* species produce gas from D-glucose.

Most *Enterobacter* species or biogroups are negative for the following tests: H_2S production from thiosulfate, phenylalanine deaminase, tetrathionate reduction, tributyrin and corn oil hydrolysis, and β-glucuronidase.

Bacteriophage and bacteriocin typing Phage typing has been used for *E. cloacae*. Gaston (1987b) isolated 76 phages active against *E. cloacae* from sewage. Of these, 26 phages were selected after numerical taxonomic analysis of their reaction patterns on 92 *E. cloacae* strains. This system has been tried on 384 isolates, 94% of which were susceptible to at least one phage. 325 phage susceptibility patterns were observed. Reproducibility of patterns was 100% when duplicate testing was on the same day, but the

reproducibility was only 40% when duplicate testing was done after 18 months (Gaston, 1987a).

Three different schemes have been developed for typing *E. cloacae* strains by susceptibility to bacteriocins. Freitag and Friedrich (1981) were able to type 51 of 65 strains (78%) which were assigned to 23 bacteriocin types designated A through X (type Y contained non-typable isolates). Traub et al. (1982) found 9% of 256 *E. cloacae* isolates to be bacteriocinogenic. Bacteriocins were produced by 16 strains after induction by mitomycin. A total of 308 isolates from various clinical sources were studied and 79% fell into 52 bacteriocin types leaving 21.4% of the isolates untypable. Bauernfeind and Petermüller (1984) found 132 of 149 isolates to produce bacteriocins. With a set of eight bacteriocin-producing strains, typability of 134 clinical isolates was 96.3%. A total of 44 different bacteriocin types could be distinguished. Only 11 (8. 2%) of the isolates fell into the largest bacteriocin type.

Antigenic structure *E. cloacae* serotyping schemes have been devised. Sakazaki and Namioka (1960) distinguished 53 O- and 56 H-antigens in agglutination tests ; 170 isolates were distributed among 79 serotypes. Bacterial suspensions had to be boiled in order to be agglutinated by the O-antisera. Unfortunately, epidemiological investigations using this serotyping scheme have not been reported.

Gaston et al. (1983) devised a serotyping scheme based on heat-stable somatic (O) antigens. 28 antisera including 11 absorbed sera were used, thus defining 28 O-serogroups. Of 300 clinical isolates from 66 hospitals, 78% were typable, 11% were not agglutinated by any of the sera, and 11% were autoagglutinable in saline.

Pathogenicity *Enterobacter* species are found in the natural environment including water, sewage, vegetables, and soil. The increased prevalence of *Enterobacter* spp. as nosocomial pathogens may be due to a greater resistance to disinfectants and antimicrobial agents than that of other members of the *Enterobacteriaceae*. Contaminated medicinal agents can be sources of outbreaks. Maki and Martin (1975) showed that *E. cloacae*, *E. agglomerans* and *Serratia marcescens* multiplied better in 5% dextrose solution at 25°C than did other members of the *Enterobacteriaceae*.

The pathogenicity of *Enterobacter* spp. has been reviewed by Sanders and Sanders (1997). Most *Enterobacter* bacteremias are acquired in the hospital. *E. cloacae* predominates, followed by *E. agglomerans*, *E. sakazakii*, and others. 14–53% of bacteremias that involve *Enterobacter* spp. are polymicrobial. *Enterobacter* bacteremias occur in patients with severe underlying diseases. Cases involving previously healthy individuals can be caused by administration of a contaminated medical product. The mortality rate is similar to that due to other enteric bacilli. A study of patients undergoing cardiac surgery suggested that *E. cloacae* might be more virulent than *E. aerogenes* (*Klebsiella mobilis*). Among patients colonized by *E. cloacae*, 26% developed an infection, whereas only 7% of those colonized by *K. mobilis* developed an infection.

In the lower respiratory tract, *Enterobacter* spp. have been involved in asymptomatic colonization of respiratory secretions, purulent bronchitis, lung abscess, pneumonia, and empyema. Pneumonia occurs in lung transplant recipients, patients with severe underlying diseases, and in elderly persons who are institutionalized.

Enterobacter spp. are a common cause of nosocomial infections of surgical wounds and burns. Other infections involving the skin and soft tissues are cellulitis, fasciitis, abscesses, emphysema, and myositis. Endocarditis due to *Enterobacter* spp. occurs in intravenous drug abusers and individuals with prosthetic valves.

Enterobacter spp. have been involved in biliary sepsis. Bacteremias after hepatic transplantation or endoscopic retrograde cholangiopancreatography, hepatic gas gangrene, fulminant emphysematous cholecystitis, acute suppurative cholangitis, and peritonitis following small intestine obstruction have been reported.

Urinary tract infections due to *Enterobacter* spp. range from asymptomatic bacteriuria to pyelonephritis and urosepsis. *Enterobacter* meningitis, ventriculitis, brain abscess, and infections near foreign bodies have been reported. *Enterobacter* spp. are infrequently the cause of postoperative endophthalmitis, but consequences are devastating (loss of vision or eye). Occasionally, severe septic arthritis, osteomyelitis, infections of multiple bones and joints in infants and children, vertebral osteomyelitis, bilateral hip infections and prosthetic hip infections due to *Enterobacter* spp. have been reported (reviewed by Sanders and Sanders, 1997).

Other species in the *E. cloacae* complex (*E. dissolvens*, *E. asburiae*, *E. hormaechei*) may have been reported as *E. cloacae* due to taxonomic uncertainties. *E. hormaechei* has been isolated from wounds, sputum, and blood (O'Hara et al., 1989). An outbreak of *E. hormaechei* infection and colonization among vulnerable, low-birth-weight premature infants has been described (Wenger et al., 1997). In our opinion, the *E. hormaechei* phenotype as described by O'Hara et al. (1989) is a biogroup of a larger genomic species. Thus, the strains that caused an outbreak in Marseille hospitals were identified as belonging to the *E. hormaechei* genomic species, although they resembled *E. cloacae* in classical biochemical tests (Davin-Regli et al., 1997). Our observation is that most *E. cloacae* that cause nosocomial infections belong to the *E. cloacae* group III/ *E. hormaechei* genomic species (unpublished data).

E. asburiae strains were isolated from clinical specimens, mostly urine, respiratory tract, feces, wounds, and blood (Brenner et al., 1986). The clinical significance of this organism is not known.

Enterobacter dissolvens has not yet been reported in clinical specimens and has only been recovered from environmental sources. *E. dissolvens* was first isolated by Rosen (1922) from diseased corn and is found in rotting cornstalks.

The role of *E. sakazakii* in pathology and as a contaminant has been reviewed by Nazarowec-White and Farber (1997). Little is known about the presence of *E. sakazakii* in the environment. *E. sakazakii* has frequently been found in powdered substitutes for breast milk in many countries together with other enteric bacteria. It has also been recovered from liquid formula and milk subjected to ultra high temperature (UHT) processing. Numerous reports of *E. sakazakii* meningitis and generalized sepsis have been reported in neonates. Powdered infant formula was implicated in several cases. Even when death did not occur, severe complications or sequelae were observed (brain abscesses, necrotizing enterocolitis, hydrocephalus). There have been only three reports of *E. sakazakii* infections in adults (an ulcer in the foot of a diabetic patient, urosepsis in an elderly patient, and bacteremia) (Nazarowec-White and Farber, 1997).

E. cancerogenus (*E. taylorae*) has only rarely been associated with human infections. Septicemia and urinary tract infections in patients with underlying diseases have been observed. Wound infections after severe trauma or crush injuries have been described. Some of them were chronic or unresolving infections. An environmental source was suspected (Abbott and Janda, 1997).

E. nimipressuralis has not yet been reported in clinical specimens and has only been recovered from environmental sources.

E. amnigenus is mainly found in water, but some strains were isolated from clinical specimens such as respiratory tract, wound, or feces (Farmer et al., 1985a).

E. gergoviae occurs in a variety of environmental sources such as water, cosmetics and clinical sources in France, Africa, and the United States (Richard et al., 1976; Brenner et al., 1980b). Multiply drug resistant strains have been found in urine samples during an infection outbreak (Richard et al., 1976).

Pathogenesis Adhesive properties may be important in the establishment or maintenance of bacterial infections. Adhesins are often also hemagglutinins (HA) and may or may not be located on fimbriae. Most strains of *Enterobacter amnigenus*, *E. cloacae*, and *E. sakazakii* produce a mannose-sensitive hemagglutinin (MS-HA) associated with type-1 fimbriae, i.e., thick, channeled fimbriae having an external diameter of 7–8 nm. These fimbriae can be coated by type-1 fimbrial antiserum against *E. cloacae* 035 but not by type-1 fimbrial antiserum against *Klebsiella pneumoniae* K55/1 (Adegbola and Old, 1983b). No other hemagglutinin and fimbrial type has been observed in these species.

Seven of eight *E. gergoviae* strains produce a mannose-resistant *Klebsiella*-like hemagglutinin (MR/K-HA) that agglutinates tanned ox erythrocytes and is associated with type-3 fimbriae, i.e., thin, non-channelled fimbriae having an external diameter 4–5 nm. These fimbriae can be coated with type-3 fimbrial antiserum against *K. oxytoca* K70/1 (Adegbola and Old, 1983b). No other hemagglutinin and fimbrial type has been observed in this species.

All *E. cloacae* strains tested could adhere to and invade HEp-2 cells (Keller et al., 1998). Only two of 14 *E. cloacae* isolates adhered to the human cell line Intestine-407 (Livrelli et al., 1996).

The outer membrane protein OmpX plays a role in the invasion of rabbit ileal tissue by *E. cloacae*. Invasiveness varies directly with the level of OmpX in the outer membrane (De Kort et al., 1994).

Iron is essential for bacterial growth. In the human body, iron is complexed to carrier molecules such as transferrin (in the serum) or lactoferrin (in milk and other secretions), or sequestered within cells (in heme proteins). When growing under iron-limiting conditions, potentially pathogenic *Enterobacteriaceae* produce high affinity systems to solubilize and import the required iron. These iron-chelating compound produced are mostly of two sorts, phenolate (e.g., enterochelin) and hydroxamate (aerobactin) siderophores (Payne, 1988). All strains of *E. cloacae*, *E. gergoviae*, and *E. sakazakii* tested by Reissbrodt and Rabsch (1988) produced enterochelin. Some strains of all these species produced aerobactin. Aerobactin was first isolated from a strain of *K. mobilis* (then *"Aerobacter aerogenes"*) (Gibson and Magrath, 1969). Aerobactin and cloacin DF13 bind to the same receptor sites located in the outer membrane (Van Tiel-Menkveld et al., 1982). Aerobactin is encoded by a relatively simple genetic system that has been extensively characterized. Only four genes are required for synthesis of aerobactin (Carbonetti and Williams, 1984). *E. cloacae* harbor a relatively large conjugative plasmid encoding susceptibility to cloacine DF13 as well as production and uptake of aerobactin (Krone et al., 1985).

Most clinical strains of *E. cloacae* examined have been resistant to serum bactericidal activity and could produce aerobactin and mannose-sensitive hemagglutinin (Keller et al., 1998).

Antibiotic sensitivity The antibiotic sensitivity of *Enterobacter* spp. has been reviewed by Sanders and Sanders (1997). *E. cloacae* is naturally resistant to ampicillin, cephalothin and other older cephalosporins, and cefoxitin. Most strains are sensitive to ureidopenicillins and carboxypenicillins. Strains are not very sensitive to cefamandole and cefuroxime but are more sensitive to expanded-spectrum cephalosporins and aztreonam. The highest sensitivity is to carbapenems (e.g., imipenem). Most strains are sensitive to aminoglycosides and ciprofloxacin. The sensitivity to trimethoprim-sulfamethoxazole is variable.

All species of *Enterobacter* examined to date produce a chromosomally encoded group 1 β-lactamase. This enzyme is produced only at low levels (noninducible) by *E. gergoviae* and some strains of *E. sakazakii*, which explains the greater sensitivity of these strains to ampicillin, older cephalosporins, and cefoxitin. The enzyme is inducible in *E. cloacae*, *E. cancerogenus*, *E. asburiae*, and most strains of *E. sakazakii*, and these strains are resistant to ampicillin, older cephalosporins, and cefoxitin (Sanders and Sanders, 1997).

The *ampD* mutation in *E. cloacae* stably derepresses the chromosomal β-lactamase, which is then produced at high levels, causing the strains to be resistant to extended-spectrum cephalosporins, broad-spectrum penicillin, and aztreonam. These mutants remain sensitive to carbapenems and cefepime. However, additional mutations involving permeability through the outer envelope can lead to resistance to these agents. A chromosomally encoded carbapenemase has been reported (Sanders and Sanders, 1997).

Strains can acquire plasmid-borne resistance determinants encoding group 2b (TEM-1, TEM-2, or SHV-1) or group 2d (OXA-1) β-lactamases. Strains carrying such plasmids are susceptible to extended-spectrum cephalosporins and β-lactamase inhibitor-β-lactam drug combinations. Resistance to extended-spectrum cephalosporins can also be acquired with plasmids encoding group 2be β-lactamase (extended-spectrum β-lactamases).

Resistance to aminoglycosides is due to the production of one or more aminoglycoside-inactivating enzymes. The most frequently occurring enzymes are acetylating enzymes AAC(3)-II, AAC(6′), AAC(3)-III, AAC(3)-I, and AAC(3)-V. A nucleotidylating enzyme, ANT(2′′), may also occur (Sanders and Sanders, 1997).

Ecology Nitrogen-fixing strains of *E. cloacae* have been isolated from the roots of dryland and wetland rices (Ladha et al., 1983). The nitrogen-fixing *E. cloacae* strains isolated by Bally et al. (1983) belong to *E. cloacae* genomic group 5 (P.A.D. Grimont, unpublished observations). A strain identified as *E. cloacae* suppresses sporangium germination in *Pythium ultimum* (the cause of seed and root diseases of a wide variety of plants). This is achieved by a competitive utilization of fatty acids present in the exudate of germinating seeds (Van Dijk and Nelson, 2000). Some strains identified as *E. cloacae* are endophytic symbionts of corn (Hinton and Bacon, 1995).

A strain identified as *E. asburiae* is a cotton endophyte that is able to colonize internal tissues of different plant species (Quadt-Hallmann and Kloepper, 1996).

E. nimipressuralis has been described as being the causal agent of wetwood in elm trees (Carter, 1945). *Enterobacter cancerogenus* (as *Erwinia cancerogena*) has been described as the causal agent of a canker disease of poplar (*Populus* species). *Enterobacter pyrinus* causes a pear brown leaf spot disease in Korea (Chung and Cho, 1993).

Enrichment and Isolation Procedures

All media designed for the isolation of *Enterobacteriaceae* can be used for the isolation of *Enterobacter* species: MacConkey agar, Drigalski lactose agar, Hektoen agar, deoxycholate lactose citrate agar, etc. *Enterobacter* can also grow on media for general use, such as blood agar, nutrient agar, tryptic soy agar, bromocresol purple lactose agar, etc.

There are no selective media for *Enterobacter* species. The requirements for devising a selective medium are (i) a precise and stable delineation of the genus (or species, if the medium is to be species specific), (ii) known common properties within the genus (or species) which are uncommon outside the genus (or species), and (iii) a clinical, public health or special need for such selective medium. Due to pending changes in the delineation of the genus *Enterobacter* and the species *E. cloacae*, conditions (i) and (ii) are not met. Except for some epidemiological studies, the selective isolation of *Enterobacter* spp. is rarely necessary, since the presence of *Enterobacter* spp. in pluribacterial habitats (feces, throat, skin) is clinically meaningless. The public health significance of *Enterobacter* spp. in water or foods is uncertain. Isolation of *Enterobacter* spp. from clinical specimens is done either by direct plating on blood agar, tryptic soy agar or nutrient agar (e.g., pus, urine) or by plating after prior growth in tryptic soy broth or nutrient broth (blood, pus, cerebrospinal fluid).

Maintenance Procedures

Strains are initially grown on tryptic soy agar at their optimum temperature and then stabbed into a medium[1] designed for maintenance of *Enterobacteriaceae* and related organisms. The cultures are stored at room temperature in a dark, dry place. Cultures may be also preserved by freeze-drying. Freeze-drying is the best procedure for preservation of pigmented strains.

Procedures for Testing Special Characters

Procedures for carbon source utilization (using Biotype-100 strips), glucose oxidation, gluconate- and 2-ketogluconate dehydrogenase, Voges–Proskauer (Richard's modification), and β-xylosidase tests are given in the chapter on *Serratia* in this volume.

Differentiation of the genus *Enterobacter* from other genera

Because the genus is not monophyletic and may be split further, differentiation of the genus should be at the species level.

Taxonomic Comments

The history of some species of the genus *Enterobacter* can be confusedly traced to the end of the nineteenth century. *"Bacillus lactis aerogenes"* was isolated by Escherich (1885) from milk and renamed *"Bacillus aerogenes"* by Kruse (1896) and *"Aerobacter aerogenes"* by Beijerinck (1900b). Differentiation of this organism from Friedländer's bacillus (now *Klebsiella pneumoniae*) was not clear before 1955, and most authors considered *"B. lactis aerogenes"* or *"Aerobacter aerogenes"* as nonmotile, or as containing motile and nonmotile strains (Grimbert and Legros, 1900; Edwards and Fife, 1955). This led Edwards and Fife (1955) to state that *"A. aerogenes"* strains were in fact *Klebsiella* strains.

"Bacterium cloacae" was described by Jordan (1890) and transferred to a new genus *"Cloaca"* as *"Cloaca cloacae"* by Castellani and Chalmers (1920). *Bergey's Manual* (Bergey et al., 1923) transferred this species to the genus *"Aerobacter"* as *"A. cloacae"*. Because *"Aerobacter aerogenes"* was indistinguishable (at that time) from *Klebsiella pneumoniae*, it was proposed that the species *"A. aerogenes"* disappear (Edwards and Fife, 1955) although disappearance of the type species (*"A. aerogenes"*) implied disappearance of the genus (*"Aerobacter"*).

A significant step forward occurred when Møller (1955) devised simple methods for testing amino-acid decarboxylases. The *"Cloaca"* group, being arginine-positive, could now easily be distinguished from the *Klebsiella* group (arginine-negative) by biochemical tests. This lead to the finding of motile strains of the *"Cloaca"* group which were arginine-negative and produced gas from inositol and glycerol (Hormaeche and Munilla, 1957). These were called "Cloaca B" (arginine-positive strains forming the "Cloaca A group"). After reexamination of many cultures with decarboxylase tests, Hormaeche and Edwards (1958) redefined the genus *"Aerobacter"* to include two species, *"A. aerogenes"* ("Cloaca B") and *"A. cloacae"* ("Cloaca A"). The type species was reaffirmed to be *"A. aerogenes"*.

In an attempt to avoid confusion resulting from the reclassification in the genus *Klebsiella* of many nonmotile strains previously labeled *"A. aerogenes"*, Hormaeche and Edwards (1960a, b) proposed a new genus, *Enterobacter*, as a substitute for *"Aerobacter"*. This genus was then composed of *E. cloacae* (type species) and *E. aerogenes*. The Judicial Commission of the International Committee on Nomenclature of Bacteria placed the name *Enterobacter* on the list of conserved names (Judicial Commission, 1963).

The genus *Erwinia* has long been a depository for plant-associated members of the *Enterobacteriaceae*. Several species of this genus were found phenotypically similar to *Enterobacter* species (*Erwinia herbicola*, *Erwinia ananas**, *Erwinia uredovora*, *Erwinia milletiae*, *Erwinia dissolvens*, *Erwinia nimipressuralis*) (Lelliott, 1974; Lelliott and Dickey, 1984) or highly related to *Enterobacter* species by DNA hybridization (*Erwinia dissolvens*, *Erwinia nimipressuralis*) (Steigerwalt et al., 1976).

The other *Enterobacter* species have been described after delineation by DNA–DNA hybridization. DNA–DNA hybridization has allowed bacteriologists to modify the circumscription of some species and to find synonymies.

Enterobacter taylorae was described based on clinical strains closely related to *E. cloacae* (Farmer et al., 1985b). The species was later found synonymous to *Enterobacter cancerogenus* by both DNA relatedness and phenotypic characters (Grimont and Ageron, 1989).

Early DNA relatedness studies on *E. cloacae* (Steigerwalt et al., 1976) showed the genomic heterogeneity of this nomenspecies. In work done in our laboratory (Grimont and Grimont, 1992; and unpublished) on 49 strains previously identified as *E. cloacae*, 45 strains fell into five DNA relatedness groups (1 to 5). Group 1 contained only three strains, including the type strains of *E. cloacae* and *E. dissolvens*. DNA from these type strains showed some divergence (ΔT_m values of 4.5°C). In spite of a search for similar strains in our large laboratory collection, no other strain of group 1 could be found. Group 2 contained seven strains, including

1. Maintenance medium (g/liter): Bacto-peptone (Difco), 10.0, NaCl, 5.0: Bacto-agar (Difco), 10.0; pH 7.4. The medium should be dispensed into small (9.5–10 × 90 mm) screw-capped tubes.

**Editorial Note:* Mergaert et al. (1993) transferred *Erwinia ananas* to the genus *Pantoea* as *P. ananas*; the name was corrected to *Pantoea ananatis* by Trüper and De' Clari (1997).

reference strain CDC 1347-71 (Steigerwalt et al., 1976). Group 3 contained 15 strains, including the type strain of *Enterobacter hormaechei*. This group was slightly heterogeneous with ΔT_m values ranging from 0.0–4.0°C. Group 4 contained 17 strains and could be split into three subgroups based on ΔT_m values (0.0–2.5°C within subgroup and 4.0–6.5°C between subgroups). Subgroup 4a contained the type strain of *Enterobacter asburiae*. Group 5 (earlier referred to as group 6 (Bouvet et al., 1989)) contained three nitrogen-fixing strains.

Three problems are raised by this study. The first problem involves *E. hormaechei*. A group of similar strains (Enteric group 75), which differ phenotypically from *E. cloacae*, was genomically distinct from the type strain of *E. cloacae* and thus described as a new species, *E. hormaechei* (O'Hara et al., 1989). In our group 3, we found only one strain phenotypically similar to *E. hormaechei*. All other strains of group 3 fit the classical definition of *E. cloacae* (Richard, 1984). Group 3 strains (including *E. hormaechei*) produce an active glucose dehydrogenase, and this property differentiates group 3 from the other groups in the *E. cloacae* complex (Bouvet et al., 1989; P.A.D. Grimont, unpublished observations). Group 3 should be named *E. hormaechei*, and the species definition should be emended.

The second problem involves *E. asburiae*. Enteric group 17 differed from *E. cloacae* by some phenotypic features and DNA hybridization showed Enteric Group 17 (*E. asburiae*) to be distinct from the type strain of *E. cloacae*. Our subgroup 4a is genomically and phenotypically identical with *E. asburiae*. However, subgroups 4b and 4c are less differentiable from *E. cloacae* by phenotypic characteristics. Group 4 (including subgroups 4a, 4b, and 4c) should be called *E. asburiae*, and the species definition should be amended.

The third problem is in fact the cause of the other two. The choice of the neotype strain for *E. cloacae* was unfortunate. This strain was isolated from cerebrospinal fluid, which is not the usual habitat of what is currently known as *E. cloacae*. There are two possible solutions: either the nomenclatural rules are followed, in which case *E. cloacae* (our group 1) will disappear from routine clinical laboratory work (because most strains presently labeled *E. cloacae* are in fact *E. hormaechei* or *E. asburiae*), or the type strain can be changed and a new one designated from our group 2 (which can be encountered in clinical microbiology, although less frequently than *E. hormaechei* and *E. asburiae* genomic species).

Some species that were included in the genus *Enterobacter* are now excluded; these are discussed below.

The group of strongly proteolytic strains named *"Aerobacter liquefaciens"* by Grimes and Hennerty (1931) was included in the genus *Enterobacter* by Edwards and Ewing (1972). The species was transferred to the genus *Serratia* as *S. liquefaciens* by Bascomb et al. (1971).

A group of strains named *Hafnia* by Møller (1954) was included in the genus *Enterobacter* as *E. hafniae* (Ewing and Fife, 1968). This species appeared in the 8th edition of the *Bergey's Manual* (Buchanan and Gibbons, 1974) as *Hafnia alvei*. Steigerwalt et al. (1976) favored the separation of *Hafnia alvei* from the genus *Enterobacter* based on DNA relatedness studies.

E. aerogenes is closer to *Klebsiella pneumoniae* (about 55% DNA relatedness) than to *E. cloacae* (about 45% relatedness) (Brenner et al., 1972c; Steigerwalt et al., 1976). In a numerical taxonomy study (Bascomb et al., 1971), *E. aerogenes* was so similar to the genus *Klebsiella* that transfer of this species to the genus *Klebsiella* was proposed. Since the name *"K. aerogenes"* had been used for bacteria that are indistinguishable from *K. pneumoniae* by DNA relatedness, the name *K. mobilis* was proposed for *E. aerogenes* (Bascomb et al., 1971). The species is treated in the chapter on the genus *Klebsiella* as *K. mobilis*.

E. agglomerans is a very complex group of environmental bacteria that may cause opportunistic infections. The name covers many (20–40) genomic groups (Brenner et al., 1988b) or phenons (Gavini et al., 1983b; Verdonck et al., 1987). In addition to this diversity, strains of the *E. agglomerans* complex are not closely related to *E. cloacae* (the type species of the genus *Enterobacter*) by DNA relatedness. Some groups in this complex have been placed in new genera (*Rahnella aquatilis*, *Ewingella americana*, *Leclercia adecarboxylata*). A new genus, *Pantoea*, has been proposed for several groups of the *E. agglomerans* complex. These are treated in the chapter on the genus *Pantoea*.

Enterobacter intermedius (originally named *E. intermedium*; corrected by von Graevenitz (1990)) was a Voges–Proskauer positive species found in water and unpolluted soil (Izard et al., 1980). However, it has an *rrs* sequence identical to that of *Kluyvera cochleae*. Furthermore, DNA–DNA hybridization has shown the type strains of *E. intermedius* and *K. cochleae* to be 99% related with a thermal instability of hybridized molecules of 1.5°C (D.J. Brenner, personnal communication).

Further Reading

Janda, J.M. and S.L. Abbott. 1998. The Enterobacteria, Lippincott-Raven, Philadelphia. pp. 387.

Differentiation of the species of the genus *Enterobacter*

Tables BXII.γ.220 and BXII.γ.221 give the characteristics differentiating the species and unnamed genomic groups in the genus *Enterobacter*.

List of species of the genus Enterobacter

1. **Enterobacter cloacae** (Jordan 1890) Hormaeche and Edwards 1960b, 72[AL]. Nom. Cons. Opin. 28, Jud. Comm. 1963, 38 (*Bacillus cloacae* Jordan 1890, 836.)
clo.a' cae. L. n. *cloaca* a sewer; L. gen. n. *cloacae* of sewer.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Tables BXII.γ.220, BXII.γ.221 and BXII.γ.222. Several DNA hybridization groups compose this species. Some of these DNA groups can be identified to named species (*E. dissolvens*, *E. asburiae*, or *E. hormaechei*) with different circumscriptions than originally published. Occurs in water, sewage, soil, meat, and hospital environments and on the skin and in the intestinal tracts of man and animals as a commensal. May cause nosocomial infections

The mol% G + C of the DNA is: 52–54 (T_m).

Type strain: ATCC 13047, CIP 60.85, DSM 30054, JCM 1232, LMG 2783, NCTC 10005.

GenBank accession number (16S rRNA): AJ417484.

This strain is very close by DNA–DNA hybridization to the type strain of *E. dissolvens* and may not properly represent the strains routinely identified as *E. cloacae*.

TABLE BXII.γ.220. Characteristics of the species of the genus *Enterobacter*[a]

Characteristic	*E. cloacae* complex[b]	*E. amnigenus*	*E. cancerogenus*	*E. cowanii*	*E. gergoviae*	*E. kobei*	*E. nimipressuralis*	*E. pyrinus*	*E. sakazakii*
Motility (36°C)	d	+	+	+	+	+	+	+	+
Yellow pigment	−	−	−	d	−	−	−	−	+
Urea hydrolyzed	−	−	−	−	+	d	−	+	−
Indole production	−	−	−	−	−	−	−	−	d
β-Xylosidase test	+	+	−	+	−	+	+	ND	+
Methyl red	d	−	−	+	−	ND	+	ND	−
Voges–Prokauer	d	+	+	+	+	−	+	+	+
Growth in KCN	+	+	+	+	−	+	+	+	+
Gelatin hydrolysis at 22°C	(d)	−	−	−	−	−	−	−	−
Deoxyribonuclease (25°)	−	−	−	−	−	−	−	−	(+)
Lysine decarboxylase	−	−	−	−	+	−	−	d	−
Arginine dihydrolase	+	+	+	−	−	+	+	−	+
Ornithine decarboxylase	+	+	+	−	+	d	+	+	+
Phenylalanine deaminase	−	−	−	−	−	−	−	−	d
Glucose dehydrogenase	D	−	−	+	+	ND	−	ND	+
Gluconate dehydrogenase	−	−	−	−	+	ND	−	+	−
Growth at 41°C	+	−	ND	+	+	+	−	ND	+
Esculin hydrolysis	d	+	−	+	+	d	+	+	+
Acetate	d	−	−	+	+	d	ND	ND	+
Acid from:									
Adonitol	d	−	−	−	−	−	−	−	−
L-Arabinose	+	+	+	+	+	+	+	+	+
D-Arabitol	d	−	−	−	+	−	ND	ND	−
Cellobiose	+	+	+	+	+	+	+	+	+
Dulcitol	D	−	−	+	−	d	−	−	−
meso-Erythritol	−	−	−	−	−	ND	−	ND	−
Glycerol	d	−	d	+	+	d	ND	+	−
myo-Inositol	d	−	−	−	−	d	d	+	(+)
Lactose	d	d	−	+	d	+	+	−	+
Maltose	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+
Melibiose	d	+	−	+	+	+	+	+	+
α-Methylglucoside	+	d	d	−	−	+	+	−	+
Mucate	d	+	d	d	−	d	+	ND	−
Raffinose	+	+	−	+	+	+	d	−	+
L-Rhamnose	d	+	+	+	+	+	+	+	+
Salicin	D	+	+	+	+	+	+	+	+
D-Sorbitol	+	−	−	+	−	+	+	−	−
Sucrose	+	+	−	+	+	+	−	+	+
Trehalose	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+
Utilization of:									
cis-Aconitate	d	−	+	+	d	−	−	(d)	+
trans-Aconitate	d	−	+	+	−	−	−	(d)	d
Adonitol	D	−	−	−	−	−	−	−	−
4-Aminobutyrate	−	−	−	−	+	−	−	−	+
5-Aminovalerate	−	−	−	−	+	−	−	−	−
D-Arabitol	D	−	−	−	+	−	−	+	−
Benzoate	−	−	−	−	d	−	−	−	−
Citrate	+	+	+	+	+	+	+	−	+
m-Coumarate	−	−	−	−	+	−	−	−	−
Dulcitol	D	−	−	+	−	+	−	−	d
L-Fucose	D	−	+	−	d	−	−	−	−
Gentisate	−	−	−	(+)	d	−	−	−	−
Histamine	−	−	−	−	+	−	−	−	−
3-Hydroxybenzoate	−	−	−	−	d	−	−	−	−
4-Hydroxybenzoate	−	−	−	−	+	−	−	−	−
3-Hydroxybutyrate	d	−	−	−	d	−	−	−	−
myo-Inositol	D	−	−	−	d	−	−	−	d
5-Ketogluconate	−	−	−	−	+	−	−	+	−
2-Ketoglutarate	d	−	d	−	d	−	−	d	−
Lactose	(+)	+	(d)	(+)	(+)	+	+	−	+
Lactulose	d	d	−	(+)	d	+	−	−	+
D-Lyxose	D	+	−	+	−	−	+	−	−
D-Malate	d	−	d	(+)	d	+	d	−	(d)
Malonate	d	−	(d)	−	d	d	−	−	(d)
Maltitol	+	+	−	−	−	+	+	−	+
D-Melibiose	D	+	−	+	+	+	+	−	+
1-*O*-Methyl-α-galactoside	D	+	−	+	+	+	+	−	+
3-*O*-Methyl-D-glucose	D	−	+	−	−	v	−	−	−
1-*O*-Methyl-α-D-glucoside	+	d	−	−	−	+	+	−	+

(*continued*)

TABLE BXII.γ.220. *(cont.)*

Characteristic	*E. cloacae* complex[b]	*E. amnigenus*	*E. cancerogenus*	*E. cowanii*	*E. gergoviae*	*E. kobei*	*E. nimipressuralis*	*E. pyrinus*	*E. sakazakii*
Mucate	d	+	+	+	−	+	+	−	−
Palatinose	+	+	−	−	−	+	+	−	+
Phenylacetate	D	−	+	−	+	−	+	−	−
L-Proline	+	d	+	+	+	+	+	−	+
Protocatechuate	−	−	−	−	+	−	−	+	−
Putrescine	D	−	+	−	d	−	−	+	+
Quinate	−	−	−	−	+	−	−	−	−
D-Raffinose	d	+	−	+	+	+	d	−	+
L-Rhamnose	D	+	+	+	+	+	+	+	+
D-Saccharate	+	+	+	+	−	+	+	+	−
D-Sorbitol	+	−	−	+	−	+	+	−	−
Sucrose	+	+	−	+	+	+	−	+	+
D-Tagatose	d	−	−	d	−	−	d	+	−
meso-Tartrate	−	−	−	−	d	−	−	−	−
Tricarballylate	−	−	−	−	d	−	−	−	−
Tryptamine	−	−	−	−	−	−	−	−	−
D-Turanose	d	(d)	−	−	−	+	d	−	d
L-Tyrosine	d	−	−	−	d	−	−	−	−

[a] +, 90–100% strains positive in 1–2 days; (+), 90–100% strains positive in 1–4 days; −, 90–100% strains negative in 4 days; d, positive or negative in 1–4 days; (d), positive or negative in 3–4 days; D, test used to differentiate species within a complex; ND, no data.

[b] The *E. cloacae* complex includes *E. cloacae*, *E. dissolvens*, *E. hormaechei*, and *E. asburiae*.

TABLE BXII.γ.221. Phenotypic properties of genomic groups within the *Enterobacter cloacae* complex[a]

Characteristic	Genomic group or subgroup[b] 1	2	3	4a	4b	4c	5
Glucose dehydrogenase	−	−	+	−	−	−	−
Motility	+	+	+	−	d	+	+
Malonate test	+	+	+	−	−	d	+
Esculin hydrolyzed	d	(d)	(d)	+	+	+	+
Utilization of:							
Adonitol	−	−	d	−	−	−	−
D-Arabitol	−	−	d	−	−	−	−
Dulcitol	−	d	d	−	−	d	+
Fucose	−	−	d	−	−	−	−
D-Galacturonate	+	d	+	+	+	+	+
myo-Inositol	+	+	d	+	+	+	+
Lyxose	d	−	+	+	+	d	+
D-Melibiose	+	+	d	−	+	+	+
3-Methylglucose	−	−	d	−	−	−	−
Phenylacetate	d	+	+	−	+	+	+
Putrescine	d	+	−	+	−	+	−
D-Raffinose	+	+	d	d	d	+	+
L-Rhamnose	+	+	+	−	d	−	+
D-Sorbitol	+	+	d	+	+	+	+
Xylitol	−	−	(d)	−	−	−	−

[a] +, 90–100% strains positive in 1–2 days (utilization tests) or in 1 day (other tests); (+), 90–100% strains positive in 1–4 days; −, 90–100% strains negative in 4 days; d, positve or negative in 1–4 days; (d), positive or negative in 3–4 days.

[b] The type strain of *E. dissolvens* and the present type strain of *E. cloacae* are in genomic group 1; the type strain of *E. hormaechei* is in genomic group 3, and the type strain of *E. asburiae* is in genomic group 4, subgroup 4a.

TABLE BXII.γ.222. Utilization of substrates by biogroups of genomic group 3 of the *E. cloacae* complex[a]

Utilization of:	Biogroup[b] 3a	3b	3c	3d	3e	3f	3g
Adonitol	−	−	−	−	−	+	+
D-Arabitol	−	−	−	−	−	+	+
Fucose	d	+	+	−	+	+	+
Methyl-α-D-galactoside	−	+	+	+	−	+	+
3-Methylglucose	+	−	+	−	−	+	−
D-Melibiose	−[b]	+	+	+	−	+	+
D-Raffinose	−	+	+	+	+	+	+
D-Sorbitol	−	+	+	+	+	+	+

[a] +, all strains positive in 1–2 days; −, strains negative in 4 days; d, positive or negative in 1–4 days.

[b] Five strains representing *E. hormaechei* (including the type strain) and received from the Centers for Disease Control corresponded to biogroup 3a.

2. **Enterobacter amnigenus** Izard, Gavini, Trinel and Leclerc 1981b, 37[VP]

am.ni′ge.nus. L. adj. *amnigenus* coming from water.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Isolated from water and rarely from clinical samples.

The mol% G + C of the DNA is: 60 (Bd).

Type strain: ATCC 33072, CCUG 14182, CIP 103169, DSM 4486, JCM 1237, LMG 2784, NCTC 12124..

GenBank accession number (16S rRNA): AB004749.

3. **Enterobacter asburiae** Brenner, McWhorter, Kai, Steigerwalt and Farmer III 1988b, 220[VP] (Effective publication: Brenner, McWhorter, Kai, Steigerwalt and Farmer III 1986, 1117.)

as.bur′i.ae. N.L. gen. n. *asburiae* named in honor of Marie Alyce Fife-Asbury, an American bacteriologist.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are included in Tables BXII.γ.220 and BXII.γ.221.

The type strain belongs in DNA group 4a in the *E. cloacae* complex.

The mol% G + C of the DNA is: 55 (T_m).

Type strain: ATCC 35953, CIP 103358, JCM 6051, NCTC 12123.

GenBank accession number (16S rRNA): AB004744.

4. **Enterobacter cancerogenus** (Urosevic 1966) Dickey and Zumoff 1988, 373[VP] *Erwinia cancerogena* Urosevic 1966, 500.)

can.cer.o′ge.nus. L. *cancer* crab, the disease cancer; L. v. *gigno* to produce; L. masc. adj. *cancerogenus* cancer-inducing.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220.

Isolated from cankers in poplars (*Populus* species) in

Czechoslovakia and in clinical specimens. *Enterobacter taylorae* (Farmer et al. 1985b; type strain ATCC 35317 = CDC 2126-81) is a junior synonym of *E. cancerogenus* (Grimont and Ageron, 1989).

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 33241, CCUG 25231, CFBP 4167, CIP 103787, ICMP 5706, LMG 2693, NCBBP 2176.

GenBank accession number (16S rRNA): Z96078.

5. **Enterobacter cowanii** Inoue, Sugiyama, Kosako, Sakazaki and Yamai 2001, 1619^{VP} (Effective publication: Inoue, Sugiyama, Kosako, Sakazaki and Yamai 2000, 419.)
co.wa′ ni.i. M.L. gen. n. *cowani* of Cowan, named after S.T. Cowan, British bacteriologist.

The cell morphology is as given for the genus. Colonies on nutrient agar are often yellow pigmented. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Isolated from diverse clinical specimens but clinical significance is unknown. May be found in foods.

The mol% G + C of the DNA is: 53 (HPLC).

Type strain: 88-76, CIP 107300, JCM 10956.

6. **Enterobacter dissolvens** (Rosen 1922) Brenner, McWhorter, Kai, Steigerwalt and Farmer III 1988b, 220^{VP} (Effective publication: Brenner, McWhorter, Kai, Steigerwalt and Farmer III 1986, 1119 (*"Phytomonas dissolvens"* Rosen 1922, 497; *Erwinia dissolvens* (Rosen 1922) Burkholder 1948a, 472.)
dis.sol′ vens. L. part. adj. *dissolvens* dissolving.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are included in Tables BXII.γ.220 and BXII.γ.221. Presently, it is not possible to phenotypically differentiate *E. dissolvens* from *E. cloacae*.

The type strain belongs in DNA group 1 in the *E. cloacae* complex. Originally isolated from diseased corn.

The mol% G + C of the DNA is: 54 (Bd).

Type strain: ATCC 23373, CIP 105586, ICMP 1570, JCM 6049, LMG 2683, NCBBP 1850.

GenBank accession number (16S rRNA): AJ417485, Z96079.

The type strain belongs in the *Enterobacter cloacae* complex.

7. **Enterobacter gergoviae** Brenner, Richard, Steigerwalt, Asbury and Mandel 1980b, 1^{VP}
ger.go′ vi.ae. M.L. gen. n. *gergoviae* of Gergovie Highland, France.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Occurs in various environmental sources such as cosmetics or water. Has also been recovered from clinical specimens.

The mol% G + C of the DNA is: 60 (Bd).

Type strain: ATCC 33028, CIP 76.1, DSM 9245, JCM 1234, LMG 5739, NCTC 11434.

GenBank accession number (16S rRNA): AB004748.

8. **Enterobacter hormaechei** O'Hara, Steigerwalt, Hill, Farmer, Fanning and Brenner 1990, 105^{VP} (Effective publication: O'Hara, Steigerwalt, Hill, Farmer, Fanning and Brenner 1989, 2048.)
hor.ma.e′ che.i. M.L. gen. n. *hormaechei* of Hormaeche, named after Estenio Hormaeche, Uruguayan microbiologist.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Tables BXII.γ.220 and BXII.γ.221. Oxidation of glucose into gluconate in the absence of PQQ is a characteristic of the *E. hormaechei* genomic group. Isolated from clinical specimens.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 49162, CCUG 27126, CIP 103441.

GenBank accession number (16S rRNA): AJ417450.

9. **Enterobacter kobei** Kosako, Tamura, Sakazaki and Miki 1997, 915^{VP} (Effective publication: Kosako, Tamura, Sakazaki and Miki 1996, 264.)
kobe.i. M.L. gen. n. *kobei* pertaining to the city of Kobe, Japan.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Isolated from diverse clinical specimens but clinical significance is unknown. May be found in foods.

The mol% G + C of the DNA is: 53 (T_m).

Type strain: ATCC BAA-260, CIP 105566, DSM 13645, JCM 8580.

10. **Enterobacter nimipressuralis** (Carter 1945) Brenner, McWhorter, Kai, Steigerwalt and Farmer 1988b, 220^{VP} (Effective publication: Brenner, McWhorter, Kai, Steigerwalt and Farmer III 1986, 1119 (*"Erwinia nimipressuralis"*) Carter 1945, 423; Dye 1969a, 83.)
ni.mi.pres.su.ra′ lis. L. adv. *nimis* overmuch; L. n. *pressura* pressure; M.L. adj. *nimipressuralis* with excessive pressure.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Reported as the causative agent of "wetwood" disease in elm trees (Carter, 1945).

The mol% G + C of the DNA is: 55 (Bd).

Type strain: ATCC 9912, CIP 104980, ICMP 1577, JCM 6050, NCPPB 2045.

GenBank accession number (16S rRNA): Z96077.

11. **Enterobacter pyrinus** Chung, Brenner, Steigerwalt, Kim, Kim and Cho 1993b, 161^{VP}
pyr′ i.nus. L. n. *pyrus* pear; L. suff. *-inus* belonging to; L. adj. *pyrinus* from pears.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Isolated from brown leaf spot lesions on pear trees in Korea.

The mol% G + C of the DNA is: 57–61 (T_m).

Type strain: ATCC 49851, CFBP 4168, CIP 104019, DSM 12410, ICMP 12530.

12. **Enterobacter sakazakii** Farmer, Asbury, Hickman and Brenner 1980a, 575^{VP}
sa.ka.za′ ki.i. M.L. gen. n. *sakazakii* of Sakazaki; named after the Japanese bacteriologist, Riichi Sakazaki.

The cell and colonial morphology are as given for the genus. Physiological and nutritional characteristics are presented in Table BXII.γ.220. Occurs in the environment and in foods. May contaminate milk powder and subsequent nutritional preparations and then cause generalized infection (septicemia, meningitis) in newborns.

The mol% G + C of the DNA is: 57 (T_m).

Type strain: ATCC 29544, CCUG 14558, CIP 103183, DSM 4485, JCM 1233, LMG 5740, NCTC 11467.

GenBank accession number (16S rRNA): AB004746.

Genus XIII. **Erwinia** *Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209[AL] emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1*

LYSIANE HAUBEN AND JEAN SWINGS

Er.wi'ni.a. M.L. fem. n. *Erwinia* named after Erwin F. Smith.

Straight rods, 0.5–1.0 × 1.0–3.0 μm; occur singly or in pairs. Gram negative. Motile by peritrichous flagella. Facultatively anaerobic, but anaerobic growth by some species is weak. Optimum temperature, 27–30°C; maximum temperature for growth is 40°C. Oxidase negative. Catalase positive. **Pectinase negative.** Acid is produced from fructose, D-galactose, D-glucose, and sucrose but not from adonitol, arabitol, dextrin, dulcitol, inulin, maltose, starch, and tagatose. Utilize fumarate, D-galactose, gluconate, D-glucose, glycerol, β-methylglucoside, malate, and succinate, but not L-arabitol, benzoate, butanol, methanol, oxalate, propionate, or sorbose as carbon- and energy-yielding sources. Utilize L-alanine, L-glutaminic acid, glycylglycine, and L-serine, but not kynureninic acid and trigonelline, as nitrogen sources. Sensitive to chloramphenicol, furazolidone, nalidixinic acid, oxytetracyline, and tetracycline. Do not possess arginine dihydrolase, caseinase, pectinase, phenylalanine deaminase, or urease. Associated with plants as pathogens, saprophytes, or constituents of the epiphytic flora.

The species of the genus *Erwinia* comprise a distinct phylogenetic group, as determined by 16S rRNA gene sequence comparisons, and are characterized by 14 generic signature nucleotides (Table BXII.γ.223).

Erwinia species cause plant diseases that include mainly blights and wilts. The pathogen usually starts to cause damage in the vascular tissue and then spreads throughout the plant. Ingress by the pathogen generally occurs through natural openings and wounds.

The mol% G + C of the DNA is: 49.8–54.1.

Type species: **Erwinia amylovora** (Burrill 1882) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209 emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Micrococcus amylovorus* Burrill 1882, 134.)

FURTHER DESCRIPTIVE INFORMATION

Gas production is comparatively weak or absent. Decarboxylases for arginine, lysine, or ornithine cannot be detected by Møller's method (Møller, 1955). Formation of putrescine occurs when the amino acids are decarboxylated under aerobic conditions (Zherebilo and Gvozdyak, 1976). Glutamic acid is not decarboxylated. Lipases are rarely produced. Additional characteristics of the species of the genus are given in Tables BXII.γ.223, BXII.γ.224, and BXII.γ.225.

Fermentation end products from glucose are CO_2 and different combinations of succinate, lactate, formate, and acetate; some strains form 2,3-butanediol and some ethanol (White and Starr, 1971). Starch is not hydrolyzed beyond dextrins.

Sequence analyses on 16S rDNA of the species of the genus *Erwinia* by Kwon et al. (1997) and Hauben et al. (1998a) are in good agreement and place the genus *Erwinia* within the *Enterobacteriaceae*, closely related to the genera *Pantoea*, *Pectobacterium*, *Brenneria*, and *Enterobacter* (Fig. BXII.γ.201; Table BXII.γ.223).

Burrill (1882) was the first to attribute the cause of a plant disease to a bacterium, which he named *"Micrococcus amylovorus"*. In successive taxonomic studies, the bacterium was renamed *"Bacillus amylovorus"* (Trevisan, 1889a), *"Bacterium amylovorus"* (Chester, 1897), *"Bacterium amylovorum"* (Serbinov, 1915), and finally *Erwinia amylovora* (Winslow et al., 1920). *Erwinia* species cause plant diseases that include blights, die back, leaf spots, wilts, and crown rot. *Erwinia tracheiphila* overwinters in the bodies of cucumber beetles (*Diabrotica vittata* Fabr. and *D. duodecimpunctata* Oliv.) (Leach, 1964).

Phytopathogenicity of *Erwinia rhapontici* and *Erwinia persicina** may be due to the release of proferrorosamines (Feistner et al., 1997).

ENRICHMENT AND ISOLATION PROCEDURES

In general, *Erwinia* can be easily isolated. The affected plant material is washed in tap water, followed by sterile water, and dried by paper toweling. Surface sterilization (3 min in 1:10 dilution of 5.25% active sodium hypochlorite) is sometimes detrimental for isolation. Affected tissue is removed from a young lesion or the edge of older necrotic areas by a sterile scalpel; the tissue is crushed in sterile water, saline, or buffer solution and is streaked onto a solid medium, such as nutrient agar or yeast dextrose chalk agar (YDC) (0.5% yeast extract, 1% dextrose, 3% calcium carbonate) (Dye, 1968). The isolation of *E. tracheiphila* is more easily accomplished by aseptically cutting the affected stem, placing the two cut stem surfaces together, and then gently pulling apart, removing a portion of the threads of bacteria and placing them in nutrient broth or onto a solid medium (Burkholder, 1960b). The delicate growth of *E. tracheiphila* will appear in 3–4 d; frequent transfer is necessary, but virulence may be reduced or lost after repeated transfers.

The isolation of some *Erwinia* species can be facilitated by use of selective-differential media, but such media are usually not necessary. *E. amylovora* will grow on MS medium (Miller and Schroth, 1972) and produce characteristic colonies. Later, Schroth and Hildebrand (1980) substituted D-mannitol by D-sorbitol in the MS medium for the isolation of *E. amylovora*. The medium of Crosse and Goodman (1973)[1] or CCT medium[2] can also be used for *E. amylovora*. A soluble pink pigment is produced by *E. persicina* and *E. rhapontici* (Hao et al., 1990) on medium containing 1% casitone, 0.3% yeast extract, 0.5% NaCl, and 0.7% agar.

MAINTENANCE PROCEDURES

Stock cultures of *Erwinia* species should be grown on standard media of choice at 25–30°C until good growth occurs. The cul-

**Editorial Note: Erwinia persicina* was originally known as *Erwinia persicinus*; the name was corrected by Euzéby (1998).

1. Crosse and Goodman's medium consists of sucrose, 160 g; nutrient agar, 12 g; 0.1% crystal violet in absolute ethanol, 0.8 ml; 0.1% cycloheximide, 20 ml; 380 ml distilled water.

2. CCT medium consists of sucrose, 100 g; D-sorbitol, 10 g; 1% tergitol anionic 7, 30 ml; 0.1% crystal violet in absolute ethanol, 2 ml; nutrient agar (Difco), 23 g; 970 ml distilled water; after autoclaving add 2 ml 1% (w/v) thallium nitrate and 50 mg cycloheximide (Ishimaru and Klos, 1984).

TABLE BXII.γ.223. Diagnostic characteristics of the genera *Erwinia*, *Brenneria*, *Enterobacter*, *Pantoea*, and *Pectobacterium*[a]

Characteristic	*Erwinia*	*Brenneria*	*Enterobacter*	*Pantoea*	*Pectobacterium*
Biochemical characteristics:					
Citrate (Simmons)	nd	nd	+	nd	D
Production of acetoin	d	d	D	+	+
Arginine dihydrolase	−	−	nd	nd	D
Arginine decarboxylase	nd	−	nd	nd	−
Lysine decarboxylase	nd	−	D	nd	−
Ornithine decarboxylase	nd	−	+	nd	−
Esculine hydrolase	D	D	nd	nd	+
Caseinase	−	−	nd	nd	D
Pectinase	−	D	nd	nd	D
Phenylalanine deaminase	−	−	−	nd	D
Urease	−	D	nd	nd	−
mol% G + C of DNA	49–54	50–56	52–60	55–57	50–56
Acid production from:					
Adonitol	−	−	D	nd	−
N-acetylglucosamine	D	d	nd	nd	+
Inulin	−	nd	nd	nd	D
D-Lyxose	D	nd	nd	nd	−
Maltose	−	D	nd	+	D
D-Mannitol	D	+	nd	+	
D-Mannose	D	+	nd	+	+
L-Rhamnose	D	+	nd	nd	+
D-Ribose	D	+	nd	+	+
Salicin	D	+	nd	nd	+
Trehalose	D	D	nd	+	D
D-Xylose	D	D	nd	+	
Utilization of carbon sources:					
Acetate	D	nd	nd	nd	+
4-Aminobutyrate	nd	nd	nd	+	−
Arbutin	D	+	nd	nd	+
Citrate	+	D	+	nd	+
Maltose	nd	D	nd	nd	D
Mannose	D	+	nd	nd	+
L-Proline	D	nd	nd	nd	nd
Sucrose	nd	d	nd	nd	+
Trehalose	nd	D	nd	nd	d
16S rDNA signature nucleotides:					
E. coli numbering position:					
379	C	G	C	C	C
384	G	C	S	G	G
408	A	R	A	A	G
434	Y	Y	T	T	C
593	Y	T	C	T	Y
598	C	Y	C	C	T
599	R	R	G	A	G
638	Y	Y	C	T	C
639	G	R	G	G	A
646	G	R	G	G	A
839	C	G	C	C	C
847	G	C	G	G	G
848	H	Y	M	A	C
987	G	A	R	A	R
988	G	C	S	C	C
989	C	T	T	T	T
1216	G	A	A	A	A
1217	C	R	S	G	G
1218	C	T	Y	T	T
1308	C	T	T	C	T
1329	G	A	A	G	A

[a]For symbols see standard definitions; nd, not determined.

tures can be maintained for a maximum of 3 weeks in a refrigerator (4–5°C).

For long-term preservation, erwiniae can be successfully stored as lyophilized cultures usually suspended in a filter sterilized mixture of 200 ml horse serum (Oxoid SR035C) to which 1675 mg nutrient broth (Oxoid) and 20 g glucose in 67 ml distilled water is added. Strains have also been stored in liquid nitrogen and in glycerol at −80°C (broth + 15% glycerol). Regular viability controls are recommended.

TABLE BXII.γ.224. Diagnostic characteristics of *Erwinia* species[a]

Characteristic	1. *E. amylovora*	2. *E. billingiae*	3. *E. mallotivora*	4. *E. persicina*	5. *E. psidii*	6. *E. rhapontici*	7. *E. tracheiphila*
Pink diffusible pigment	−	nd	−	+	−	+	−
Mucoid growth	+	nd	+	nd	nd	+	−
Growth in KCN broth	−	nd	−	nd	nd	+	−
Growth in 5% NaCl	nd	nd	−	nd	nd	+	−
Growth factors required	+	nd	+	nd	nd	−	+
$NO_3^- \rightarrow NO_2^-$	−	+	−	+	−	+	−
Levan	+	nd	+	nd	+	nd	+
H_2S from cysteine	−	nd	v	nd	+	+	+
Production of acetoin	+	+	+	+	−	v	d
Esculin hydrolase	+	nd	−	nd	nd	nd	nd
Gelatinase	+	−	v	nd	v	−	−
Acid production from:							
N-Acetylglucosamine	nd	+	nd	−	nd	nd	+
Amygdalin	nd	−	nd	−	nd	nd	+
L-Arabinose	d	+	−	−	+	+	v
D-Cellobiose	−	−	−	+	−	+	−
Dulcitol	−	nd	v	−	+	d	−
Esculin	−	nd	−	nd	nd	+	−
Gentiobiose	+	+	−	−	nd	nd	−
D-Gluconate	nd	+	nd	−	nd	nd	nd
α-Methylglucoside	−	−	−	−	−	d	v
β-Methylglucoside	+	nd	+	+	+	+	−
Glycerol	−	−	−	−		+	v
Myo-inositol	−	nd	v	−	+	+	−
Inulin	−	nd	−	−	nd	+	−
Lactose	−	−	−	+	−	+	−
Lyxose	nd	−	nd	−	nd	+	nd
Maltose	−	nd	−	nd	nd	+	−
D-Mannose	−	+	+	nd	+	+	v
Melezitose	−	−	−	−	nd	+	−
Melibiose	−	−	−	+	nd	+	−
Raffinose	−	−	v	+	−	+	−
L-Rhamnose	−	+	−	+	+	+	v
D-Ribose	+	nd	+	+		+	−
Salicin	−	nd	v	nd	+	+	−
Starch	−	nd	−	−	nd	+	−
Trehalose	+	+	v	+	−	+	−
Xylitol	nd	−	nd	−	nd	+	nd
D-Xylose	−	+	v	−	−	d	−
Utilization of carbon sources:							
Acetate	+	−	+	+	−	nd	+
L-Arabinose	−	nd	−	+	+	nd	v
D-Arabitol	−	+	−	−	−	−	−
Arbutin	nd	+	−	+	nd	nd	+
D-Cellobiose	nd	nd	−	+	nd	nd	−
Citrate	+	−	+	+	nd	+	+
Formate	+	nd	−	nd	nd	+	d
Galacturonic acid	−	nd	−	nd	nd	d	−
L-Glutamate	−	nd	−	+	+	nd	−
Glycerol	nd	nd	nd	+	nd	nd	−
L-Histidine	−	nd	−	+	−	nd	−
DL-Lactate	+	nd	−	+	nd	+	−
Lactose	nd	nd	nd	+	nd	nd	−
Malonate	−	−	−	nd	−	+	−
D-Mannose	−	nd	+	+	+	nd	v
Melibiose	+	nd	−	+	−	nd	−
Proline	−	nd	−	+	−	nd	−
Raffinose	−	nd	−	+	−	nd	−
Tartrate	−	nd	−	nd	−	d	v
D-Xylose	−	nd	+	nd	−	nd	−
Utilization of nitrogen sources:							
L-Isoleucine	+	nd	−	nd	nd	nd	nd
Threonine	+	nd	−	nd	nd	nd	nd
Tryptamine	nd	nd	−	nd	nd	+	−
Xanthin	nd	nd	+	nd	nd	nd	−
Sensitivity toward:							
Furazolidone	+	nd	−	nd	nd	nd	nd

[a]For symbols see standard definitions; nd, not determined.

TABLE BXII.γ.225. Additional characteristics of *Erwinia* species[a]

Characteristic	1. *E. amylovora*	2. *E. billingiae*	3. *E. mallotivora*	4. *E. persicina*	5. *E. psidii*	6. *E. rhapontici*	7. *E. tracheiphila*
Levan	+	nd	+	nd	+	nd	+
Production of indole	−	−	−	nd	−	−	−
Oxidase	−	−	−	−	−	−	−
Catalase	+	+	+	+	+	+	+
Alkaline phosphatase	nd	nd	nd	+	nd	nd	nd
Arginine dihydrolase	−	−	−	−	−	−	−
Caseinase	−	nd	−	−	−	−	−
Arylsulfatase	nd	nd	nd	−	nd	nd	nd
Esterase	nd	nd	nd	−	nd	nd	nd
β-Galactosidase	nd	+	nd	nd	nd	nd	+
β-Glucosidase	nd	nd	nd	+	nd	nd	nd
β-Glucuronidase	nd	nd	nd	−	nd	nd	nd
Lysine decarboxylase	nd	−	nd	nd	nd	nd	−
Nucleoside phosphotransferase	nd	nd	nd	−	nd	nd	nd
Pectinase	−	−	−	−	−	−	−
Phenylalanine deaminase	−	nd	−	−	−	−	−
Starch hydrolase	−	nd	−	nd	−	nd	−
Tetrathionate reductase	nd	−	nd	−	nd	nd	nd
Tween 80 hydrolase	nd	−	nd	−	nd	nd	nd
Urease	−	−	−	−	−	−	−
Acid production from:							
Adonitol	−	−	−	−	−	−	−
Dextrin	−	nd	−	−	−	−	−
Dulcitol	−	−	−	−	−	−	−
Erythritol	nd	−	nd	nd	nd	nd	−
Fructose	+	+	+	+	+	+	+
Fucose	nd	−	nd	−	nd	nd	−
D-Galactose	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+
Inulin	−	−	−	−	−	−	−
2-Ketogluconate	nd	nd	nd	−	nd	nd	nd
5-Ketogluconate	nd	nd	nd	−	nd	nd	nd
Maltose	−	nd	−	−	−	−	−
Mannitol	v	+	+	nd	+	+	v
α-Methylmannoside	nd	−	nd	−	nd	nd	−
β-Methylxyloside	nd	−	nd	nd	nd	nd	−
Sorbitol	d	nd	v	+	+	+	v
Sorbose	nd	−	nd	−	nd	nd	−
Starch	−	−	−	−	−	−	−
Sucrose	+	nd	+	+	+	+	+
Tagatose	−	−	−	−	−	−	−
D-Turanose	nd	−	nd	−	nd	nd	nd
Utilization of carbon sources:							
N-Acetylglucosamine	nd	nd	nd	+	nd	nd	nd
Aconitate	nd	nd	nd	+	nd	nd	nd
Adipate	nd	nd	nd	−	nd	nd	−
Adipinic acid	nd	nd	nd	nd	nd	nd	−
Adonitol	nd	nd	nd	nd	nd	nd	−
DL-α-Alanine	nd	nd	nd	+	nd	nd	nd
Amygdalin	nd	nd	nd	+	nd	nd	nd
D-Arabinose	nd	nd	nd	−	nd	nd	nd
L-Arabitol	−	nd	−	−	−	−	−
L-Aspartate	nd	+	nd	+	nd	nd	nd
Benzoate	−	nd	−	−	−	−	−
Betaine	nd	nd	nd	nd	nd	nd	−
Butanol	−	nd	−	−	−	−	−
Butyrate	nd	nd	nd	−	nd	nd	nd
Caprylate	nd	nd	nd	−	nd	nd	nd
Cysteine	nd	nd	nd	−	nd	nd	nd
Dextrin	nd	nd	nd	nd	nd	nd	−
Dulcitol	nd	nd	nd	−	nd	nd	−
Erythritol	nd	nd	nd	+	nd	nd	nd
Esculin	nd	nd	nd	+	nd	nd	nd
Ethanol	nd	nd	nd	nd	nd	nd	−
Ethylene glycol	nd	nd	nd	nd	nd	nd	−
Fructose	nd	nd	nd	+	nd	nd	nd
Fucose	nd	nd	nd	−	nd	nd	nd
Fumarate	+	nd	+	+	+	+	+
Galactose	+	nd	+	+	+	+	+
Gluconate	+	nd	+	+	+	+	+
Glucose	+	nd	+	+	+	+	+
DL-Glycerate	nd	nd	nd	+	nd	nd	nd

(*continued*)

TABLE BXII.γ.225. *(cont.)*

Characteristic	1. *E. amylovora*	2. *E. billingiae*	3. *E. mallotivora*	4. *E. persicina*	5. *E. psidii*	6. *E. rhapontici*	7. *E. tracheiphila*
Glycerol	+	nd	+	+	+	+	+
L-Glycine	nd	nd	nd	−	nd	nd	nd
L-Histamine	nd	nd	nd	−	nd	nd	nd
myo-Inositol	nd	nd	nd	+	nd	nd	nd
Inulin	nd	nd	nd	−	nd	nd	nd
2-Ketogluconate	nd	nd	nd	+	nd	nd	nd
5-Ketogluconate	nd	nd	nd	+	nd	nd	nd
Lactulose	nd	nd	−	nd	nd	nd	−
L-Leucine	nd	nd	nd	−	nd	nd	nd
L-Lysine	nd	nd	nd	−	nd	nd	nd
Lyxose	nd	nd	nd	−	nd	nd	−
Malate	+	+	+	+	+	+	+
Malonic acid	nd	nd	nd	nd	nd	nd	−
Maltose	nd	nd	+	+	nd	nd	+
D-Mannitol	nd	nd	nd	+	nd	nd	nd
Melezitose	nd	nd	nd	−	nd	nd	nd
Methanol	−	nd	−	−	−	−	−
β-Methylglucoside	+	nd	+	+	+	+	+
Naphthalene	nd	nd	nd	nd	nd	nd	−
L-Ornithine	nd	nd	nd	−	nd	nd	nd
Oxalate	−	nd	−	−	−	−	−
Pectinic acid	nd	nd	nd	nd	nd	nd	−
Propanol	nd	nd	nd	nd	nd	nd	−
Propionate	−	nd	−	−	−	−	−
Propionic acid	nd	nd	nd	nd	nd	nd	−
L-Rhamnose	nd	nd	nd	+	nd	nd	nd
Ribose	nd	nd	nd	+	nd	nd	+
Salicin	nd	nd	nd	+	nd	nd	+
L-Serine	nd	nd	nd	+	nd	nd	nd
Sorbinic acid	nd	nd	nd	nd	nd	nd	−
Sorbitol	+	nd	nd	+	nd	nd	+
Sorbose	−	nd	−	−	−	−	−
Spermine	nd	nd	nd	−	nd	nd	nd
Starch	nd	nd	nd	−	nd	nd	−
Succinate	+	nd	+	+	+	+	+
Sucrose	nd	nd	nd	+	nd	nd	+
D-Tagatose	nd	nd	nd	−	nd	nd	nd
L-Threonine	nd	nd	nd	−	nd	nd	nd
Trehalose	nd	nd	nd	+	nd	nd	nd
Tryptamine	nd	nd	nd	−	nd	nd	nd
D-Turanose	nd	nd	nd	−	nd	nd	nd
Urea	nd	nd	nd	−	nd	nd	nd
L-Valine	nd	nd	nd	−	nd	nd	nd
Xylitol	nd	nd	nd	nd	nd	nd	−
Utilization of nitrogen sources:							
L-Alanine	+	nd	+	+	+	+	+
Allantoin	nd	nd	nd	nd	nd	nd	+
Ammonium chloride	nd	nd	nd	nd	nd	nd	+
Anthranilic acid	nd	nd	−	nd	nd	nd	nd
Arginine	nd	nd	nd	nd	nd	nd	+
Betaine	nd	nd	nd	nd	nd	nd	−
Choline	nd	nd	nd	nd	nd	nd	−
Citrulline	nd	nd	nd	nd	nd	nd	+
Cysteamine	nd	nd	nd	nd	nd	nd	−
Glucosamine	nd	nd	nd	nd	nd	nd	+
L-Glutaminic acid	+	nd	+	+	+	+	+
Glutathion	nd	nd	nd	nd	nd	nd	+
Glycylglycine	+	nd	+	+	+	+	+
Hydroxyproline	nd	nd	nd	nd	nd	nd	−
Kynureninic acid	−	nd	−	−	−	−	−
Leucine	nd	nd	nd	nd	nd	nd	+
L-Methionine	+	nd	nd	nd	nd	nd	nd
Phenylalanine	nd	nd	nd	nd	nd	nd	+
Quinolinic acid	nd	nd	nd	nd	nd	nd	−
Sarcosine	nd	nd	nd	nd	nd	nd	−
L-Serine	+	nd	+	+	+	+	+
Spermidine	nd	nd	nd	nd	nd	nd	−
Spermine	nd	nd	nd	nd	nd	nd	−
Thymine	nd	nd	−	nd	nd	nd	−
Trigonelline	−	nd	−	−	−	−	−
L-Tryptophan	nd	nd	+	nd	nd	nd	nd
Tyramine	nd	nd	nd	nd	nd	+	nd

(*continued*)

TABLE BXII.γ.225. *(cont.)*

Characteristic	1. *E. amylovora*	2. *E. billingiae*	3. *E. mallotivora*	4. *E. persicina*	5. *E. psidii*	6. *E. rhapontici*	7. *E. tracheiphila*
Tyrosine	nd	nd	nd	nd	nd	nd	+
Valine	nd	nd	−	nd	nd	nd	nd
Sensitivity to antibiotics:							
Amikacin	nd	nd	nd	+	nd	nd	nd
Amoxycillin	nd	nd	nd	nd	nd	+	+
Ampicillin	nd	nd	+	nd	nd	+	+
Carbenicillin	nd	nd	+	nd	nd	nd	+
Cephalexin	nd	nd	+	nd	nd	nd	+
Cephaloridine	nd	nd	+	nd	nd	nd	+
Cephalotine	nd	nd	+	nd	nd	nd	+
Chloramphenicol	+	nd	+	+	+	+	+
Clindamycin	nd	nd	nd	+	nd	nd	nd
Erythromycin	nd	nd	nd	+	nd	+	nd
Framycetine	nd	nd	nd	nd	nd	nd	+
Furazolidone	+	nd	+	+	+	+	+
Fusidinic acid	nd	nd	−	nd	nd	nd	−
Gentamicin	nd	nd	nd	+	nd	nd	nd
Kanamycin	nd	nd	+	nd	nd	nd	+
Lincomycin	nd	nd	nd	+	nd	nd	nd
Methicillin	nd	nd	nd	nd	nd	nd	−
Minocyclin	nd	nd	nd	+	nd	nd	nd
Nalidixinic acid	+	nd	+	+	+	+	+
Nitrofurantoin	nd	nd	nd	+	nd	nd	nd
Novobiocin	nd	nd	−	nd	nd	nd	nd
Oxytetracycline	+	nd	+	+	+	+	+
Penicillin G	nd	nd	nd	−	nd	nd	nd
Polymyxin B	nd	nd	nd	+	nd	nd	nd
Spectinomycin	nd	nd	+	nd	nd	nd	nd
Streptomycin	v	nd	+	+	nd	nd	nd
Sulfafurazol	nd	nd	−	nd	nd	nd	−
Tetracycline	+	nd	+	+	+	+	+

[a]For symbols see standard definitions; nd, not determined.

Differentiation of the genus *Erwinia* from other genera

Characteristics that differentiate *Erwinia* from the genera *Pectobacterium*, *Pantoea*, and *Brenneria* are given in Table BXII.γ.223. As it is very difficult to differentiate them phenotypically; genomic methods are recommended for differentiation.

Taxonomic Comments

The genus *Erwinia*, named after the phytobacteriologist Erwin F. Smith, was created in 1920 to unite all Gram-negative, fermentative, nonsporulating, peritrichously flagellated plant-pathogenic bacteria (Winslow et al., 1920). The taxonomy of the genus *Erwinia* and designation of species in the genus has been complicated by the heterogeneity of the strains included in the taxon. It has been suggested in the past that members of the genus be placed into new groupings with other members of the *Enterobacteriaceae* (Starr and Mandel, 1969; White and Starr, 1971). This concept also was supported by studies of DNA–DNA relatedness (Gardner and Kado, 1972), DNA relatedness (Brenner et al., 1974a), and DNA–DNA segmental relatedness (Murata and Starr, 1974).

Waldee (1945) suggested that *Erwinia* should be limited to pathogens (*E. amylovora*, *Erwinia salicis*, and *E. tracheiphila*) that cause necrotic or wilt diseases, utilize a restricted range of carbon compounds, and usually require organic nitrogen compounds for growth, and that the biochemically more active soft rotting pathogens (*Erwinia carotovora* and *Erwinia chrysanthemi*) should be placed in a separate genus *Pectobacterium*. Although some workers have supported this suggestion (Brenner et al., 1973b, 1974a), it was not generally accepted because some species are taxonomically intermediate between these two groups, i.e., they resemble *E. carotovora* in most of their characteristics but do not cause rots.

Taxonomic changes since the previous edition of *Bergey's Manual of Systematic Bacteriology* The species *Erwinia herbicola*, *Erwinia milletiae* (Gavini et al., 1989b), *Erwinia ananas*, *Erwinia uredovora*, and *Erwinia stewartii* (Mergaert et al., 1993) have been transferred to the genus *Pantoea*. The species *incertae sedis Erwinia dissolvens*, *Erwinia nimipressuralis* (Brenner et al., 1986), and *E. cancerogena* (Dickey and Zumoff, 1988) have been transferred to the genus *Enterobacter*. Five new *Erwinia* species and two new subspecies were created: *Erwinia alni* (Surico et al., 1996), *Erwinia billingiae* (Mergaert et al., 1999), *Erwinia cacticida* (Alcorn et al., 1991), *Erwinia persicina* (Hao et al., 1990), *Erwinia psidii* (Rodrigues-Neto et al., 1987), *E. carotovora* subsp. *odorifera* (Gallois et al., 1992), and *E. carotovora* subsp. *wasabiae* (Goto and Matsumoto, 1987).

Confusion exists about the organisms named *Erwinia carnegieana*. None of the original isolates are available and no strains have been isolated corresponding to the original description (Alcorn and Orum, 1988). The type strain of *E. carnegieana* NCPPB 439 is nonpectinolytic and non-plant pathogenic and was identified as a *Klebsiella pneumoniae* (Graham, 1964; Edwards and Ewing, 1972; Alcorn and Orum, 1988).

Recently obtained sequence data allowed estimations of phylogenetic relationships, shed a new light on these earlier viewpoints, and raised new perspectives for the taxonomy of the genus

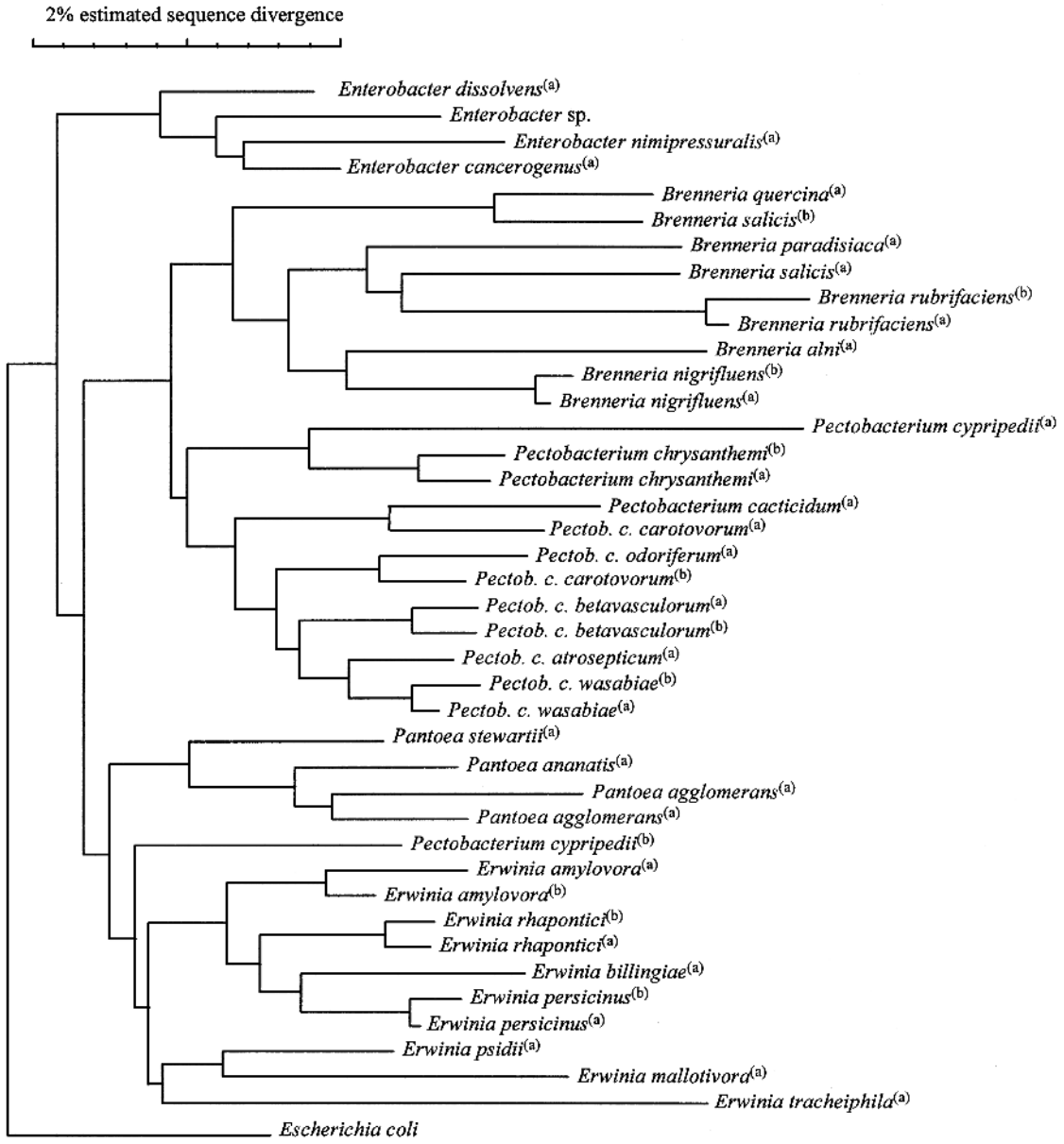


FIGURE BXII.γ.201. Neighbor-joining dendrogram depicting the estimated phylogenetic relationships among the genera *Erwinia*, *Pectobacterium*, *Brenneria*, *Pantoea*, and *Enterobacter*, based on pairwise comparisons of nearly complete 16S rDNA sequences calculated with the software package GeneCompar (Applied Maths, Kortrijk, Belgium), after removal of all unknown bases and gaps. The distance between two species is obtained by summing the lengths of the connecting horizontal branches using the scale on the top. *Pectob. c.* = *Pectobacterium carotovorum* subspecies; *(a)*: 16S rDNA sequence obtained from Hauben et al. (1998a); *(b)*: 16S rDNA sequence obtained from Kwon et al. (1997).

Erwinia. After comparison of the 16S rDNA sequences of representatives of the genus *Erwinia* with other members of the *Enterobacteriaceae*, the phylogenetic positions of the *Erwinia* species were determined, and led to the emended description of the genera *Erwinia* and *Pectobacterium* and to the description of the new genus *Brenneria* (Hauben et al., 1998a).

DIFFERENTIATION OF THE SPECIES OF THE GENUS *ERWINIA*

The characteristics of the species of *Erwinia* are given in Tables BXII.γ.223, BXII.γ.224, and BXII.γ.225. Only four strains of *E. tracheiphila* were studied, and data for this species should be treated with caution.

List of species of the genus Erwinia

1. **Erwinia amylovora** (Burrill 1882) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209[AL] emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Micrococcus amylovorus* Burrill 1882, 134.)
a.my.lo′vo.ra. Gr. n. *amylum* starch; L. v. *voro* to devour; M.L. fem. adj. *amylovora* starch-destroying.

The characteristics are as given for the genus and as listed in Tables BXII.γ.224 and BXII.γ.225.

The virulence of *E. amylovora* is often correlated with its capacity to produce extracellular polysaccharides (or EPS, e.g., amylovoran), lipopolysaccharide, a high-molecular-mass levan, a low-molecular-mass glucan (Roberts and Coleman, 1991), and levansucrase (Gross et al., 1992). Levansucrase-negative mutants show retarded development of necrotic symptoms on inoculated pear seedlings (Geier and Geider, 1993). RcsA and RcsB are two regulator proteins that interact with the promoter of the operon for amylovoran biosynthesis (Bereswill and Geider, 1997; Kelm et al., 1997). The genes *amsA* to *amsI* are required for exopolysaccharide synthesis (Bugert and Geider, 1995, 1997).

After 2–3 days at 27°C, colonies on 5% sucrose nutrient agar are typically white, domed, shining, mucoid (levan type) with radial striations, and a dense flocculent center or central ring. Nonlevan forms are isolated rarely.

Several regions of the bacterial DNA of *E. amylovora* are species specific and can be used for identification by (1) a PCR assay with primers AMSbL (5′-GCTACCAGCAGGGTGAG-3′) and AMSbR (5′-TCATCACGATGGTGTAG-3′) derived from the ams region, with an annealing temperature of 49°C (Bereswill et al., 1995); (2) a typical banding pattern derived from arbitrarily primed PCR with oligonucleotide APT1 (5′-CAGGACGCTACTTGTGT-3′), annealing temperature 32°C, from the sequence of transposon Tn5 (Bereswill et al., 1995); (3) a PCR assay with primers, primer A (5′-CGGTTTTTAACGCTGGG-3′) and primer B (5′-GGGCAAATACTCGGATT-3′), annealing at 52°C, derived from a 29-kb plasmid (pEA29) that modulates the development of fire blight (Falkenstein et al., 1989; Bereswill et al., 1992); (4) nested PCR with primers A (5′-CGGTTTTTAACGCTGGG-3′), B (5′-GGGCAAATACTCGGATT-3′), AJ75 (5′-CGTATTCACGGCTTCGCAGAT-3′), and AJ76 (5′-ACCCGCCAGGATAGTCGCATA-3′) with an annealing temperature of 52°C, and PCR-dot-blot hybridizations derived from pEA29 (McManus and Jones, 1995); and (5) a PCR assay with primers EaF (5′-GCGCAGTAAAGGGTGACAGCCCCGTACACAAAAAGGCACT-3′) and EaR (5′-CCCTAGCCGAAACAGTGCTCTACCCCCGG-3′) derived from the 23S rDNA sequence, with an annealing temperature of 72°C (Maes et al., 1996).

Repetitive element PCR (rep-PCR) (McManus and Jones, 1994) and RAPD fingerprinting (Momol et al., 1997) can distinguish *E. amylovora* strains isolated from Pomaceae from strains isolated from *Rubus* (subfamily Rosaceae). Pulsed field gel electrophoresis (PFGE) can distinguish *E. amylovora* strains of different geographical origin (Zhang and Geider, 1997).

E. amylovora produces harpins, bacterial protein elicitors that induce hypersensitive response-like necrosis when infiltrated into nonhost plants such as tobacco (Wei et al., 1992). Harpin activates a myelin basic protein kinase in tobacco leaves (Popham et al., 1995; Adam et al., 1997) and elicits active oxygen production in suspension cells (Baker et al., 1993). The secretion of harpin occurs via a type III pathway, similar to the export system of pathogenic proteins of *Yersinia* spp. (Bogdanove et al., 1996). These proteins are encoded by the *hrp* gene cluster, consisting of seven transcriptional units (Wei and Beer, 1995; Kim et al., 1997b). The *dsp* genes also affect pathogenicity but not the eliciting of a hypersensitive response (Roberts and Coleman, 1991). The expression of the *hrp* and *dsp* genes is temperature-sensitive.

Plasmid pULB113 was found in *E. amylovora*, mediating chromosomal mobilization and R-prime formation (Chatterjee et al., 1985). The self-transmissible 56-kb plasmid Ea322 is not involved in pathogenicity (Steinberger et al., 1990). pEa8.7, an 8.7-kb plasmid was found in streptomycin- and sulfonamide-resistant strains (Palmer et al., 1997). The genes *strA* and *strB* were found in streptomycin-resistant strains (Chiou and Jones, 1991, 1995; McManus and Jones, 1994).

Cells infected with bacteriophage ERA103 produce an enzyme that degrades the extracellular polysaccharide of noninfected cells (Vandenburgh et al., 1985). *E. amylovora* cells are sensitive to bacteriophage Mu, which can be used as a genetic tool (Vanneste et al., 1990).

Several monoclonal antibodies have been produced against *E. amylovora* strains (Schaad, 1979) that can be used for the detection of the bacterium in suspensions as well as in contaminated twigs, plant fluids, and fruit tissues. The following methods can be applied: (1) Slide agglutination tests: agglutination with *E. amylovora* antiserum is a common and accurate method of determination (Lelliott, 1968; Zielke et al., 1993; Arsenijevic et al., 1994); the species is serologically homogeneous and has few agglutinogens in common with related species or with the saprophytes found in diseased material. (2) Immunofluorescence techniques (IFT) (Lin et al., 1987; Zielke et al., 1993). (3) Enzyme-linked immunosorbent assays (double-antibody sandwich [DAS]-ELISA) (Laroche and Verhoyen, 1984; Lin et al., 1987; Zielke et al., 1993). The sensitivity of the three methods is approximately the same, i.e., 10^5 cells/ml.

Pantoea agglomerans and *Pseudomonas fluorescens* are antagonistic toward *E. amylovora* (Wilson et al., 1992; Wilson and Lindow, 1993; Wodzinski et al., 1994; Kearns and Hale, 1995, 1996; Mercier and Lindow, 1996).

E. amylovora causes a necrotic disease (fire blight) of most species of the Pomaceae and of some species in other subfamilies of the Rosaceae. Early in the season blossom and tips of twigs die back. Leaves shrivel and blacken and remain attached to the blackening stem that frequently crooks characteristically at the tip. The disease may progress into larger branches or even the trunk and form a canker. A *forma specialis* has been described from raspberry (*Rubus idaeus*) by Starr and Folsom (1951).

The mol% G + C of the DNA is: 53.6–54.1 (Bd).

Type strain: ATCC 15580, ICMP 1540, LMG 2024, NCPPB 683.

GenBank accession number (16S rRNA): Z96088, U80195.

2. **Erwinia aphidicola** Harada, Oyaizu, Kosako and Ishikara 1998, 1083[VP] Harada, Oyaizu, Kosako and Ishikara 1997, 354

a.phi' di.co.la. L. *aphid* the aphid; L. suff. *-cola* dweller; M.L. n. *aphidicola* aphid dweller.

Cells are Gram-negative, oxidase-negative, catalase-positive, fermentative rods (0.5–0.6 × 1.6–2.0 μm), and motile by means of peritrichous flagella. All strains grow well on nutrient agar and common laboratory media. Colonies on peptone-yeast extract agar are circular and smooth. These organisms grow well at 25, 30, and 35°C, but not at 42°C. Cells are motile. Voges–Proskauer test is positive, and cells can reduce nitrate. Cells can utilize citrate and malonate. Pigment is not produced. Indole is not produced. No gelatin liquefaction or tryptophan deaminase activity. H_2S production from sodium thiosulfate. No arginine hydrolase activity, no lysine decarboxylase activity, no ornithine decarboxylase activity, no deoxyribonuclease activity, and no urease activity. Acid is produced from the following carbohydrates: glycerol, erythritol, L-arabinose, D-ribose, D-xylose, adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, *N*-acetyl-D-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, melibiose, sucrose, trehalose, raffinose, D-xylitol, β-gentiobiose, D-lyxose, D-fucose, D-gluconate, 2-keto-D-gluconate, and 5-keto-D-gluconate.

The mol% G + C of the DNA is: unknown.

Type strain: IAM 14479.

3. **Erwinia billingiae** Mergaert, Hauben, Cnockaert and Swings 1999, 382[VP]

bil.ling' i.ae. L. gen. n. *billingiae* of Billing, named after Eve Billing, who first isolated these organisms.

The characteristics are as given for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225.

Antisera prepared against live or heat-killed cells, nonpurified or purified immunogens have been used for the differentiation or identification of *E. tracheiphila* (Elrod, 1946).

Strains were isolated from stem cankers, diseased blossoms, and immature fruits mainly of rosaceous trees, often in association with plant pathogens, and are considered as secondary invaders rather than primary pathogens.

The mol% G + C of the DNA is: 54.1–55.1 (T_m, Bd)

Type strain: LMG 2613, NCPPB 661.

GenBank accession number (16S rRNA): Y13249.

4. **Erwinia mallotivora** Goto 1976, 472[AL] emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1.

mal.lo.ti' vo.ra. M.L. n. *Mallotus* a genus of trees; L. v. *voro* to devour; M.L. adj. *mallotivora Mallotus*-destroying.

The characteristics are as described for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225.

Colonies on nutrient agar without sucrose are 1.5 mm in diameter, white, raised, transparent, and circular with smooth surfaces and entire margins after 2 d. Colonies on nutrient agar with 5% sucrose are flat, white, circular with entire margins and smooth surfaces, butyrous, and transparent after 1 d; after 4 d colonies are domed, circular, white, mucoid, and translucent, and sometimes possess radial striations.

Causes a leaf spot of Akamegashiwa, Mongolian oak (*Mallotus japonicus*). Initially, dark green spots appear on the leaves next to the veins. As the disease evolves, these spots grow and turn dark brown with a yellow border. Eventually, the leaf dies and twigs wilt.

The mol% G + C of the DNA is: 49.8–51.0 (Bd).

Type strain: ATCC 29573, ICMP 5705, LMG 2708, NCPPB 2851.

GenBank accession number (16S rRNA): Z96084.

5. **Erwinia persicina** Hao, Brenner, Steigerwalt, Kosaka and Komagata 1990, 382[VP] (*"Erwinia nulandii"*)

per.si' cin.a. M.L. adj. *persicina* peach color, because of the pinkish or peach-colored pigment of this organism.

The characteristics are as described for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225.

Strains vary in their ability to utilize adonitol, L-arginine, β-gentiobiose, and glucosamine. Resistance to ampicillin, carbenicillin, cefazolin, cephalothin, colistin, kanamycin, mydecamycin, nalidixic acid, sufisoxazole, and tobramycin varies.

Strains were isolated from, but are not pathogenic for, tomato, banana, and cucumber. Strains are pathogenic to bean pods and seeds. One *E. persicina* strain was recently isolated from a human (O'Hara et al., 1998).

The mol% G + C of the DNA is: 52–54 (T_m).

Type strain: ATCC 35998, CDC 9108-82, LMG 11254.

GenBank accession number (16S rRNA): Z96086, U80205.

Additional Remarks: The type strain was isolated from a tomato by Komagata and Okada in 1962.

6. **Erwinia psidii** Rodrigues-Neto, Robbs and Yamoshiro 1988, 328[VP] (Effective publication: Rodrigues-Neto, Robbs and Yamoshiro 1987, 348.)

psi' di.i. L. n. *Psidium* generic name.

The characteristics are as described for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225.

Pathogenic to guava (*Psidium guajava* L.) causing a dieback of twigs and branches, characterized by a collapse of the vascular tissue. In artificial inoculations, the bacterium was also pathogenic to strawberry guava (*Psidium cattleianum* Lam.), jambolana (*Eugenia jambolana* Lam.), and "melaleuca" (*Melaleuca viridiflora* Brogn. and Gris.), and to all members of the Myrtaceae family.

The mol% G + C of the DNA is: is 51.7–52.2 (T_m).

Type strain: ATCC 49406, ICMP 8426.

7. **Erwinia pyrifoliae** Kim, Gardan, Rhim and Geider 1999, 905[VP]

py.ri.fo' liae. L. gen. fem. n. *pyrifoliae* of *pyrifolia*, the species name of the host plant, the Nashi pear, *Pyrus pyrifolia*.

Cells are Gram-negative, nonsporeforming, peritrichous, straight rods. The strains are facultatively anaerobic; oxidase is not produced. Nitrates are not reduced. This species conforms to the definition of the family *Enterobacteriaceae*. Strains grow on YPDA medium (1% yeast extract, 2% polypeptone, 2% glucose, and 20 μg/ml adenine hemisulfate), producing colonies that are 2 mm after 48 h at 28°C. Colonies are circular, white, well-domed, and opaque. Glucose (dextrose) is fermented without gas production. Voges–Proskauer test is (weakly) positive. Polypectate gel is not acidified or liquefied. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are not present. Indole is not produced from tryptophan. Reducing compounds are produced from sucrose. Acid is not produced

from inulin, lactose, methyl-α-glucoside, melibiose, D-arabitol, D-arabinose, or raffinose. Acid is produced from mannitol, sorbitol, and saccharose. There is no alkalinization of malonate, citrate, or D-tartrate. β-Galactosidase is not produced. The following substrates are utilized as sole sources of carbon and energy: D-fructose, D-galactose, D-trehalose, saccharose, methyl-D-glucopyranoside, D-ribose, L-arabinose, glycerol, *myo*-inositol, D-mannitol, D-sorbitol, L-malate, *N*-acetyl-D-glucosamine, D-gluconate, succinate, fumarate, L-glutamate, and L-proline. The following substances are not utilized: D-mannose, D-sorbose, α-D-melibiose, D-raffinose, maltotriose, maltose, α-lactose, lactulose, methyl-β-galactopyranoside, D-cellobiose, β-gentiobiose, esculin, D-xylose, palatinose, α-L-rhamnose, α-L-fructose, D-arabitol, L-arabitol, xylitol, dulcitol, D-tagatose, maltitol, D-turanose, adonitol, hydroxyquinoline β-glucoside, 3-methyl-D-glucopyranoside, D-saccharate, mucate, L-tartrate, D-tartrate, *meso*-tartrate, D-malate, *trans*-aconitate, tricarballylate, D-glucuronate, D-galacturonate, keto-D-gluconate, L-tryptophan, phenylacetate, protocatechuate, *p*-hydroxybenzoate, quinate, *m*-hydroxybenzoate, benzoate, phenylpropionate, *m*-coumarate, trigonelline, betaine, putrescine, aminobutyrate, histamine, DL-lactate, caprate, caprylate, L-histidine, glutarate, DL-glycerate, aminovalerate, ethanolamine, tryptamine, itaconate, hydroxybutyrate, D-alanine, malonate, propionate, L-tyrosine, and ketoglutarate.

The pathogen was isolated from necrotic symptoms of leaves and branches of *Pyrus pyrifolia* cv. "Shingo". It is closely related to the fire blight pathogen *Erwinia amylovora* by the DNA sequence of the 16S rDNA, but distantly by its intergenic transcribed spacer region (ITS). It does not contain plasmid pEA29 (Rhim et al., 1999).

The mol% G + C of the DNA is: 52 (T_m).

Type strain: Ep16/96, CFBP 4172, CIP 106111, DSM 12163.

GenBank accession number (16S rRNA): AJ009930.

8. **Erwinia rhapontici** (Millard 1924) Burkholder 1948a, 475^{AL} emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Phytomonas rhapontica* (sic) Millard 1924, 11; *Pectobacterium rhapontici* (Millard 1924) Patel and Kulkarni 1951b, 80; *Erwinia carotovora* biovar rhapontici (Millard 1924) Dye 1969a, 93.)

rha.pon' ti.ci. M.L. n. *rhaponticum* specific epithet of *Rheum rhaponticum*, rhubarb; M.L. gen. n. *rhapontici* of rhubarb.

The characteristics are as described for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225. Produces proferrorosamine A.

Causes a crown rot of rhubarb (*Rheum rhaponticum*) that extends into the center of the root, pink grain of wheat that will show a cavity below the hilum (Roberts, 1974) and internal browning of hyacinth bulbs, and occurs epiphytically and saprophytically in lesions caused by other bacteria (Sellwood and Lelliott, 1978; Kokoskova, 1992). Rots potato, onion, and cucumber slices slowly, weakly, and erratically (Sellwood and Lelliott, 1978).

The mol% G + C of the DNA is: 51.0–53.1 (Bd).

Type strain: ATCC 29283, ICMP 1582, LMG 2688, NCPPB 1578.

GenBank accession number (16S rRNA): Z96087, U80206.

9. **Erwinia tracheiphila** (Smith 1895) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 173^{AL} emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Bacillus tracheiphilus* Smith 1895, 364.)
tra.che.i' phi.la. L. n. *trachia* the windpipe; Gr. adj. *philus* loving; M.L. adj. *tracheiphila* trachea-loving, i.e., growing in the tracheiphila of the vascular bundles.

The characteristics are as given for the genus and are further listed in Tables BXII.γ.224 and BXII.γ.225.

Antisera prepared against live or heat-killed cells, nonpurified or purified immunogens have been used for the differentiation or identification of *E. tracheiphila* (Elrod, 1946).

Grows very poorly on nutrient agar but moderately well on yeast extract glucose chalk agar (YCC) (10 g glucose, 5 g yeast extract, 30 g $CaCO_3$, 1.5% agar, 1 l distilled water) or glucose nutrient agar.

Causes a vascular wilt of *Cucurbita* species, *Cirullus lanatus*, and *Cucumis melo*. At first dull wilted areas appear on leaves. Later whole leaves and stems wilt, shrivel, and die. Infection is systemic and bacterial ooze is usually obvious at the cut ends of vascular tissues.

The mol% G + C of the DNA is: 50–52 (T_m, Bd).

Type strain: ATCC 33245, LMG 2906, NCPPB 2452.

GenBank accession number (16S rRNA): Y13250.

Genus XIV. **Ewingella** *Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1984a, 91^{VP}*
(Effective publication: Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1983a, 41)

CAROLINE M. O'HARA AND J.J. FARMER III

Ewing.el' la. M.L. dim. ending *-ella*; M.L. fem. n. *Ewingella* named to honor William H. Ewing an American bacteriologist who made many contributions to the nomenclature and classification of the families *Enterobacteriaceae* and *Vibrionaceae*.

Small rod-shaped cells, 0.6–0.7 × 1–1.8 μm, conforming to the general definition of the family *Enterobacteriaceae*. Contains the enterobacterial common antigen. Motile by 3–10 peritrichous flagella. Facultatively anaerobic. Gram negative, oxidase negative, catalase positive. **Some strains grow faster and are more active biochemically at 25°C than 36°C. Positive reactions for methyl red, Voges–Proskauer, citrate utilization (Simmons), ONPG test, nitrate reduction to nitrite, and acid production from D-glucose, D-mannitol, salicin, trehalose, D-arabitol, D-mannose, and D-galactose.** Utilize 35 of 156 carbon sources. Negative reactions for indole production, H_2S production (TSI), urea hydrolysis, phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, gelatin hydrolysis (22°C), growth in KCN, malonate utilization, acetate utilization, lipase (corn oil), DNase production, pigment production, and fermentation of sucrose, dulcitol, adonitol, *myo*-inositol, D-sorbitol, L-arabinose, raffinose, maltose, D-xylose, cellobiose, α-methyl-D-glucoside, erythritol, melibiose, and mucate. No visible gas is produced during

fermentation. Susceptible to colistin, nalidixic acid, sulfadiazine (most strains), gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, and carbenicillin (disk diffusion method on Mueller-Hinton agar); **resistant to penicillin and cephalothin**; variable susceptibility to ampicillin.

Isolated from human clinical specimens that are normally sterile such as blood and urine. Also isolated from other clinical specimens such as throat, sputum, and wounds. **Isolated from outbreaks of bacteremia, and from outbreaks of pseudobacteremia traced to contaminated collection tubes.** Probably an **opportunistic pathogen** with a low capacity to cause extraintestinal infections in humans. Occurs in water, food, and mollusks. Implicated as the cause of internal stipe necrosis of cultivated mushrooms. **A rarely isolated genus of *Enterobacteriaceae*.**

The mol% G + C of the DNA is: 53.6–55.2.

Type species: **Ewingella americana** Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1984a, 91 (Effective publication: Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1983a, 41.)

Further descriptive information

Ewingella has been isolated from a variety of clinical specimens. In CDC's culture collection, blood and wound isolates account for 22 of 60 isolates. The blood isolates were usually from pneumonia or surgery patients. Five were from an outbreak of bacteremia in the intensive care unit of a community hospital (Pien and Bruce, 1986). All patients had undergone either cardiovascular or peripheral vascular surgery prior to infection. The probable source was a contaminated ice bath used to cool syringes for cardiac output determinations. *E. americana* was cultured from the bath. Twenty "blood isolates" and 15 environmental isolates were related to an outbreak of pseudobacteremia in a pediatric hospital that was traced to cross contamination from nonsterile citrated blood collection tubes (McNeil et al., 1987a). Additional case reports of bacteremia have been described by Pien et al. (1983) and DeVreese et al. (1992). In these reports, blood isolates were from immunocompetent patients who had undergone coronary bypass surgery and cholecystectomy, respectively.

Wounds of thumb, toe, hand, finger, and leg accounted for nine of 60 isolates. One isolate was thought to be a colonizer (Bear et al., 1986) of a leg wound, but there was insufficient information to evaluate the clinical significance of the other strains. Heizmann and Michel (1991) described a patient with conjunctivitis, and *E. americana* was isolated from both eyes. Strains of *E. americana* have also been isolated from respiratory specimens, including sputum, throat, and tracheal aspirate, and from urine and ear.

Clinical significance The clinical significance of *E. americana* and its ability to actually cause infections is still being evaluated. Systematic study and more good case reports are needed. The blood cultures and case reports suggest that *Ewingella* is an opportunistic pathogen. Only one isolate has come from feces, and there is no evidence that *E. americana* can actually cause diarrhea or intestinal infections.

Other isolates Environmental isolates have been from aqueous solutions in the hospital environment, water, mollusks (Müller et al., 1995a), and mushrooms (Inglis and Peberdy, 1996; Inglis et al., 1996).

Internal stipe necrosis of mushrooms In 1996 Inglis and Peberdy found that *E. americana* was often present on the commercially cultivated mushroom *Agaricus bisporus*. In a second paper Inglis et al. (1996) presented evidence that *E. americana* caused this browning reaction known as internal stipe necrosis. This is a potentially serious disease to the mushroom industry in the United Kingdom, which has sales worth over 400 million dollars.

Isolation, identification, culture preservation, phenotypic characterization Strains of *E. americana* are not difficult to grow, and are typical *Enterobacteriaceae* in most respects. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility. Some strains of *E. americana* grow better, and are more active biochemically at 25°C than at 35°C.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the introductory chapter on *Enterobacteriaceae* gives the results for *E. americana* in 47 biochemical tests normally used for identification (Farmer et al., 1985a; Farmer, 1999). There are no genus- or species-specific tests or sequences for identification. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the introductory chapter on *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Several computer programs greatly facilitate analyzing the results. Table BXII.γ.226 may be helpful in differentiation.

Taxonomic Comments

Discovery and DNA–DNA hybridization After the work had been completed on the characterization and description of the genus *Cedecea* (Grimont et al., 1981a), there remained a group of 10 strains that were phenotypically similar to, but distinct from, *Cedecea*. The group was named "Enteric Group 40" (Farmer, unpublished data; mentioned in Grimont et al., 1983a), and it was differentiated from *Cedecea* strains by a negative lipase (corn oil) reaction and several other tests. DNA–DNA hybridization indicated that Enteric Group 40 was less than 21% related to the other *Enterobacteriaceae*. Based on these data, Grimont et al. (1983a) proposed that Enteric Group 40 be reclassified as a new genus *Ewingella*, with a single species *Ewingella americana*.

Another genus named *Ewingella* In literature searches a source of possible confusion is the genus of radiolarians also named *Ewingella* (Pessagno, 1969).

Phylogeny based on 16S rDNA sequencing An unpublished sequence of 1398 bases has been deposited by P.W. Inglis for *E. americana* strain NCPPB 3905 (PI 98) described by Inglis and Peberdy (1996) and Inglis et al. (1996), which is a mushroom strain, not the type strain. A 16S rRNA tree that includes *E. americana* can be found in the section on *Budvicia* in this *Manual*. These 16S rDNA sequencing data agree with data from DNA–DNA hybridization, and both lead to the conclusion that *Ewingella* is distinct from other genera of *Enterobacteriaceae*. In the tree, *Rahnella* is the closest relative, in agreement with the tree published by Spröer et al. (1999), which shows these two genera on a distinct branch. However, the two trees differ in the placement of other organisms.

TABLE BXII.γ.226. Differentiation of *Ewingella americana* from several other *Enterobacteriaceae*[a]

Test	*Ewingella americana*	*Cedecea davisae*	"*Enterobacter agglomerans* complex"[b]	*Rahnella aquatilis*	*Serratia marcescens*
Lipase (corn oil)	0[b]	91	0	0	98
DNase at 25°C	0	0	0	0	98
Malonate utilization	0	91	65	73	3
Gas from D-glucose	0	70	20	98	55
Gelatin liquefaction at 22°C	0	0	0	0	90
Fermentation of:					
L-Arabinose	0	0	95	100	0
D-Arabitol	87	100	50	6	0
D-Sorbitol	0	0	30	96	99
Yellow pigment production at 25°C	0	0	75	0	0

[a]Each number gives the percentage positive after 2 d incubation at 36°C (unless a different temperature is indicated) and is based on the data summarized by Farmer (2003). Most positive reactions occur within 24 h.

[b]See Farmer (2003) for a more detailed description of this "vernacular name". It is a term defined for practical identification in the clinical microbiology laboratory. It includes over a dozen DNA–DNA hybridization groups that were originally included in the species *Enterobacter agglomerans*, which is now known to be a heterogeneous species.

Further Reading

Grimont, P.A., J.J. Farmer III, F. Grimont, M.A. Asbury, D.J. Brenner and C. Deval. 1983. *Ewingella americana* gen. nov., sp. nov., a new *Enterobacteriaceae* isolated from clinical specimens. Ann. Microbiol. Inst. Pasteur (Paris) *134A*: 39–52.

McNeil, M.M., B.J. Davis, S.L. Solomon, R.L. Anderson, S.T. Shulman, S. Gardner, K. Kabat and W.J. Martone. 1987. *Ewingella americana*: recurrent pseudobacteremia from a persistent environmental reservoir. J. Clin. Microbiol. *25*: 498–500.

Pien, F.D. and A.E. Bruce. 1986. Nosocomial *Ewingella americana* bacteremia in an intensive care unit. Arch. Intern. Med. *146*: 111–112.

List of species of the genus Ewingella

1. **Ewingella americana** Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1984a, 91[VP] (Effective publication: Grimont, Farmer, Grimont, Asbury, Brenner and Deval 1983a, 41.)

a.mer.i.can' a. M.L. adj. *americana* to denote that the original 10 strains were isolated in the United States of America.

The characteristics are as described for the genus and as listed in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Occurs in human clinical specimens and the environment. May cause internal stipe necrosis of the mushroom species *Agaricus bisporus*, which is a potentially serious disease to the mushroom industry in the United Kingdom.

In the original study, Grimont et al. (1983a) noted that all 10 strains of *E. americana* were highly related by DNA–DNA hybridization. The relative binding percentages at both 60°C and 75°C were greater than 73%. For this reason a single species was defined and named. However, strain 0679-79 had a ΔT_m value of 5.5 at 60°C, which was much higher than all the other strains, which had values of 1.2–2.5.

Grimont et al. (1983a) defined two biogroups of *E. americana* based on three phenotypic differences. Biogroup 1 was defined to include nine strains, and does not ferment L-rhamnose and D-xylose, and is β-xylosidase negative. Biogroup 2 included strain 0679-79 described above, and was positive for these three tests. Phenotypically, this strain differed in other ways (Table II of Grimont et al., 1983a).

The original 10 strains (Grimont et al., 1983a) were very active biochemically at 36°C. However, other strains studied since 1983 have been biochemically more active at 25°C than at 36°C. These strains should be studied at both temperatures.

Additional studies are needed to determine if the unusual strains described above and the newly described strains from different habitats are genetically different from the type strain of *E. americana*.

The mol% G + C of the DNA is: 53.6–55.2 (T_m).

Type strain: ATCC 33852, CCUG 14506, CDC 1468-78, CIP 8194, DSM 4580, NCTC 12157.

Additional Remarks: The American Type Culture Collection includes four other strains from human clinical specimens: ATCC 33850, blood; ATCC 338514, sputum; ATCC 33853, gallbladder; ATCC 33854, thumb wound.

Genus XV. **Hafnia** *Møller 1954, 272*[AL]

Riichi Sakazaki

Haf ni.a. L. fem. n. *Hafnia* the old name for Copenhagen.

Straight rods, ~1.0 × 2.0–5.0 μm, conforming to the general definition of the family *Enterobacteriaceae*. Not encapsulated. Gram negative. **Motile by peritrichous flagella at 30°C**, but nonmotile strains may occur. Facultatively anaerobic. Oxidase negative. Catalase positive. **The majority of strains utilize citrate as a sole carbon source at 30°C** after 3–4 d of incubation. Nitrate is reduced to nitrite. H_2S is not produced in the butt of Kligler iron agar. Gelatinase, lipase, and deoxyribonuclease are not produced. Alginate is not utilized. Pectate is not decomposed. Phenylalanine deaminase is not produced. **Lysine and ornithine decarboxylase tests are positive**, but the arginine dihydrolase test is negative. D-Glucose is fermented with the production of acid and gas. **Acid is not produced from D-sorbitol, raffinose, melibiose, D-adonitol, D-arabitol, and *myo*-inositol.** The methyl red

test is usually positive at 35°C and negative at 22°C. Acetylmethylcarbinol is usually produced from D-glucose at 22–28°C but may not be produced at 35°C. Occur in the feces of humans and a wide range of animals including birds; also occur in sewage, soil, water, and dairy products. A member of the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 48–49.

Type species: **Hafnia alvei** Møller 1954, 272.

Further descriptive information

Members of *Hafnia* are able to grow at 35°C, but many of their physiological and biochemical activities at this temperature are irregular. The majority of strains are motile at 25–30°C, but may be nonmotile at 35°C. Although most strains fail to produce acetylmethylcarbinol from D-glucose at 35°C, they give a positive Voges–Proskauer reaction when incubated at 22–28°C. They produce gas from D-glucose and about 60% of them grow on Simmons citrate agar after incubation for 3–4 d at 25°C, but in many strains these reactions may be negative at 35°C. Lactose is not fermented, but plasmid-mediated lactose-positive strains may occur (Le Minor and Coynault, 1976).

Members of *Hafnia* are defined as H_2S-negative organisms. Møller (1954) and Kauffmann (1954) defined the *Hafnia* group of *Bacteria* as H_2S-producing organisms, since the majority of strains of *Hafnia alvei* slightly darken ferric chloride-gelatin medium (Kauffmann, 1954) and SIM medium (Difco). However, they fail to blacken the butt of Kligler iron agar and of triple sugar iron agar. Ewing (1960) suggested, for the H_2S test of the family *Enterobacteriaceae*, that sensitivity of the test should be placed at a certain level, and that either Kligler iron agar or triple sugar iron agar serves this purpose because each easily permits group differentiation within the family.

The serology of *Hafnia* was first studied by Stuart and Rustigian (1943), who divided their cultures of biotype 32011, the majority of which are now classified in *Hafnia* (Sakazaki and Namioka, 1957), into eight serovars. Eveland and Faber (1953) studied serologically 58 strains of biotype 32011 and reported 21 somatic and 22 flagellar antigens. Deacon (1952) also carried out a serological study on 17 cultures of *"Aerobacter cloacae"* including biotype 32011 and recognized 12 somatic and 6 flagellar antigens among the cultures. Serology of cultures that were biochemically defined as members of the genus *Hafnia* was studied by Sakazaki (1961) and Matsumoto (1963, 1964). They established an antigenic scheme of the *Hafnia* group consisting of 68 O groups and 34 H antigens. However, the scheme is no longer available, because many test strains for O and H antigens have been lost. Independent of the scheme of previous workers, Baturo and Raginskaya (1978) have published another antigenic scheme including 39 O and 35 H antigens of *H. alvei*. Intergeneric relationships of O antigens were recognized between *Hafnia* cultures and other members of the family *Enterobacteriaceae* (Stamp and Stone, 1944; Eveland and Faber, 1953; Harada et al., 1957; Sakazaki, 1961; Matsumoto, 1963, 1964; Sedlák and Slajsova, 1966). Deacon (1952) reported the diphasic variation in the H antigens of the *Hafnia* strains he studied, but Sakazaki (1961) and Matsumoto (1963) failed to confirm such variation. Some *Hafnia* strains may have K antigen and alpha antigen of Stamp and Stone (1944) (Deacon, 1952; Emslie-Smith, 1961). Sakazaki (1961) suggested that the K antigen that inhibits the O agglutination was a slime antigen.

The majority of strains of *H. alvei* are susceptible to carbenicillin, streptomycin, gentamicin, kanamycin, tetracycline, polymyxin B, and nalidixic acid, but resistant to cephalosporins and ampicillin. Washington et al. (1971) noted a definite difference between *H. alvei* and *Serratia liquefaciens* with respect to susceptibility testing of ampicillin and polymyxin B. They reported that most of the isolates of *Serratia liquefaciens* were susceptible to ampicillin at 20 μg/ml, while none of the *Hafnia* strains were. On the other hand, all of the *Hafnia* strains tested were susceptible to polymyxin B at 10 μg/ml, whereas only 6% strains of *Serratia liquefaciens* were.

A *Hafnia*-specific bacteriophage 1672 that provides a reliable tool for the identification of *Hafnia* strains was described by Guinée and Valkenburg (1968). They reported that the phage 1672 lysed all 100 strains of *H. alvei* tested, whereas it did not lyse strains of *Enterobacter*, *Klebsiella*, *Citrobacter*, *Serratia*, and *Salmonella*.

H. alvei occurs in humans and animals, including birds, and in natural environments such as soil, sewage, and water. McClure et al. (1957) described occurrence of the organisms in wild and caged birds, none of which appeared sick. Occasionally, the organisms cause illness. Kume (1962) described a case of equine abortion in which *H. alvei* was isolated from a fetus and lochia in pure culture. Gelev et al. (1990) reported epizootic hemorrhagic septicemia in rainbow trout associated with *H. alvei*.

Although it is difficult to assign a clear-cut clinical significance, *H. alvei* has been reported to cause septicemia (Englund, 1969; Mobley, 1971), respiratory tract infections (Washington et al., 1971; Klapholz et al., 1994; Fazal et al., 1997), meningitis (Mojtabaee and Siadati, 1978), abscesses (Washington et al., 1971; Agustin and Cunha, 1995), urinary tract infections (Whitby and Muir, 1961), and wound infections (Washington et al., 1971; Berger et al., 1977). In most cases, however, it has been found in mixed culture and seems to be an opportunistic pathogen that produces infections in patients with some underlying illness or predisposing factors such as diabetes, chronic renal failure, chronic obstructive pulmonary disease, malignancy, and HIV infection. Washington et al. (1971), who reviewed the epidemiology of *H. alvei* isolated in their laboratory, concluded that the majority of the isolates originated in the respiratory tract and were considered to be commensals, while a few were secondary invaders. They also reported that previous administration of ampicillin or cephalosporins was a common feature of patients who acquired the organisms nosocomially.

There has been a controversy on whether *H. alvei* is enteropathogenic. Kauffmann (1954) suggested that the organisms of the *Hafnia* group are probably nonpathogenic for humans. Matsumoto (1963) reported the isolation of this organism from 13% of stool specimens from apparently healthy individuals. Ewing (1986a) reported that "members of this species are not known to be incitants of gastroenteritis." On the other hand, earlier investigators (Stuart and Rustigian, 1943; Stuart et al., 1943; Deacon, 1952; Eveland and Faber, 1953) incriminated biotype 32011 of paracolon bacteria or non-lactose-fermenting *"Aerobacter cloacae"*, which are now classified in *Hafnia alvei*, as a causative agent of intestinal disorder. Harada et al. (1957) reported the isolation of *H. alvei* from sporadic cases of diarrheal diseases. Emslie-Smith (1961) suggested a possible role for *H. alvei* in gastroenteritis. In addition, Ratnam et al. (1979) reported the incrimination of *H. alvei* in a nosocomial outbreak of gastroenteritis. However, no conclusive evidence on enteropathogenicity of the *Hafnia* has been obtained. Albert et al. (1991, 1992) reported that strains of *H. alvei* isolated from diarrheal stools produced the attaching-effacing (AE) lesion in rabbits and HEp-2 cells resembling enteropathogenic *Escherichia coli* (EPEC), and that the virulence-associated gene *eaeA* was shared between EPEC and *H. alvei*. In addition, Ridell et al. (1995) found that *eaeA*-positive strains of

H. alvei have some characteristic properties in which they give negative reactions in the 2-ketogluconate and histidine assimilation tests and a positive reaction in the 3-hydroxybenzoate assimilation test in contrast with *eaeA*-negative strains. On the other hand, Ismaili et al. (1996) examined 11 strains of *H. alvei* isolated from children with diarrhea in Canada, and a Bangladesh *H. alvei* strain for AE lesion formation, presence of *eaeA* gene, profile of outer membrane protein extracts, chromosomal macrorestriction fragments, and plasmids. They found that, in contrast with the Bangladesh strain, which possessed the *eaeA* gene and forms the AE lesion, none of the Canadian strains had the *eaeA* gene, nor did they form the AE lesion. The outer membrane protein profile of all of the Canadian strains were identical to each other but differed from that of the Bangladesh strain. In addition, pulsed-field gel electrophoresis and plasmid profile analyses of the Canadian strains differed substantially from those of the Bangladesh strain. Their results indicate that there is heterogeneity among *H. alvei* strain factors associated with enteropathogenicity. Ridell et al. (1994) found an epidemiological association of *H. alvei* with diarrhea. *H. alvei* was isolated from 16% of adult Finnish tourists who visited Morocco and contracted diarrhea, but from 0% of tourists without diarrhea. *H. alvei* was isolated only from 2% of another group of adult Finnish patients with diarrhea. However, the authors found that those Finnish strains from diarrheal patients were negative for AE lesion formation and did not have the *eaeA* gene of EPEC. These recent papers suggest that some strains of *H. alvei* have the potential to cause diarrhea and that, although AE lesion formation is a virulence factor, mechanisms other than AE lesion formation may also be involved in the association of *H. alvei* with diarrhea.

Enrichment and Isolation Procedures

H. alvei can grow on less selective isolation media for enterobacteria such as eosin-methylene blue, MacConkey, xylose–lysine–deoxycholate, and Hektoen enteric agars. On highly selective isolation media, such as Salmonella–Shigella and deoxycholate-citrate agars, many of the *Hafnia* cultures would be inhibited. The *Hafnia* organisms may not grow on bismuth sulfite agar. Colonies of *H. alvei* on less inhibitory plating agar media are relatively large, translucent, circular, low convex, and colorless, with a smooth surface and entire edge and resemble those of *Salmonella* (*Hafnia* strains are sometimes misidentified as H_2S-negative *Salmonella*). Some strains may produce pink colonies on media containing sucrose. Rarely does a strain produce mucoid colonies. For the differential isolation of *H. alvei*, MacConkey agar containing 1% sorbitol may be useful, on which colorless colonies of *H. alvei* can be distinguished from other intestinal inhabitants, because the former is lactose- and sorbitol-negative but the latter are usually positive for these sugars.

There are no selective enrichment broth media for the isolation of *H. alvei*. Many strains of *H. alvei* may grow in selenite and tetrathionate broth media, but some strains fail to grow in those broths. Ability of *H. alvei* to grow at lower temperature may be applied for cold enrichment, which is a useful procedure for the isolation of *Yersinia* strains.

Maintenance Procedures

Stock cultures may be maintained at room temperature in a semisolid medium consisting of 1.0% Bacto-casitone (Difco), 0.3% yeast extract, 0.5% NaCl, and 0.3% agar (pH 7.0). *Hafnia* strains remain viable for up to several years without subculture if the culture is sealed with a rubber stopper or a cork soaked in hot paraffin wax. Strains may also be preserved by lyophilization.

Procedures for Testing Special Characters

The specific bacteriophage 1672 was isolated from surface water with *H. alvei* 1672 as the propagating strain using the following method (Guinée and Valkenburg, 1968). A well-dried nutrient agar plate is surface-inoculated with a fresh broth culture of the strain to be tested. After decantation of the plate at room temperature for 15 min, a drop of the undiluted phage 1672 is spotted on the plate with a Pasteur pipette and the plate is again allowed to dry. Readings are made after 16–20 h of incubation at 35°C. Clear plaques with a diameter of 1–5 mm may be produced. The phage preparation is obtained after the usual purification and will contain around 10^9 plaque-forming units/ml. The phage is not inactivated by heating at 60°C for 30 min.

The L-pyrrolidonyl aminopeptidase test is performed by dissolving 10 mg of L-pyrrolidonyl-β-naphthylamide (Sigma) in 10 ml of 95% ethanol. Dip sterile cotton swabs into the solution and rotate each swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Dry the swabs overnight at 37°C and store at −20°C. Make a heavy suspension (MacFarland no. 1) of the test culture into 0.2–0.3 ml of 0.1 M phosphate buffer (pH 7.4), then dip the reagent swab into the suspension and incubate at 37°C for 10 min. Finally, add a drop of cinnamaldehyde solution.[1] A positive result is shown by a pink or red color.

The β-glucuronidase test is performed by dissolving 20 mg of 4-methylumbelliferyl-β-D-glucuronide (Sigma) in a small volume of dimethyl sulfoxide and bringing the volume to 10 ml with 0.1 M phosphate buffer (pH 7.4). Dip sterile cotton swabs into the solution and rotate each swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Dry the swabs overnight at 37°C and store at −20°C. For testing, make a heavy suspension (MacFarland no. 1) of the test culture in 0.2–0.3 ml of 0.1 M phosphate buffer (pH 7.4). Dip the reagent cotton swab in the suspension and incubate at 37°C for 30 min. A positive reaction is indicated by development of blue fluorescence under long wave (360 μm) ultraviolet lamp.

Differentiation of the genus *Hafnia* from other genera

Hafnia are often misidentified as members of *Enterobacter* or *Serratia*. Members of the genus *Hafnia* give a positive reaction in lysine and ornithine decarboxylase tests but are negative in arginine dihydrolase test. In differentiating *Hafnia* from *Enterobacter* and *Serratia*, in which some species show the same reactions in those tests, the failure of *Hafnia* to ferment raffinose, D-sorbitol, adonitol, and *i*-inositol may be valuable. Moreover, members of *Hafnia* have no activity of pyrrolodonyl aminopeptidase, although *Enterobacter* and *Serratia* possess this enzyme. Stuart et al. (1943) and Eveland and Faber (1953) described a close similarity between paracolon biotype 32011, which is now classified in the *Hafnia*, and *Salmonella* in biochemical reactions. Like *Salmonella*, *Hafnia* cultures are positive in tests of lysine and ornithine decarboxylases and negative in tests of indole, urease, and fermentation of lactose and sucrose, as well as in the Voges–Proskauer

1. Cinnamaldehyde solution contains *p*-dimethlaminocinnamaldehyde (Sigma), 0.5 g; glacial acetic acid, 3.5 ml; ethylene glycol-1-methylester, 6.0 ml; sodium lauryl sulfate, 3.5 g; and distilled water, 90 ml. Store in a screw-capped brown container in a refrigerator.

reaction at 35°C, although *Hafnia* cultures fail to produce H_2S. In addition, Eveland and Faber (1953) and Harada et al. (1957) reported that the *Hafnia* cultures are often agglutinated by *Salmonella* O antisera. *Hafnia* cultures may also be misidentified with some non-lactose-fermenting *Escherichia coli* strains, because the former gives a negative reaction in tests of Voges–Proskauer and citrate utilization at 35°C. However, *E. coli* produces indole and β-glucuronidase, while *Hafnia* is negative in these two tests. On the other hand, *Hafnia* is occasionally misidentified with Shiga toxin-producing *E. coli* O157:H7, because both organisms fail to ferment sorbitol and to produce β-glucuronidase. In addition, some strains of *H. alvei* are agglutinated by *E. coli* O157 antiserum (Aleksič et al., 1992). *Yokenella regensburgei*, which was first considered to be *Hafnia*, is another organism to be differentiated from the *Hafnia*. *Y. regensburgei* is different from *Hafnia* in its ability to utilize citrate, and to produce acid from cellobiose, melibiose, and D-arabitol. *Y. regensburgei* cannot be lysed by the *Hafnia* specific bacteriophage. Table BXII.γ.227 indicates the differential characteristics between *Hafnia* and biochemically similar taxa.

Taxonomic Comments

The bacteria of the genus *Hafnia* have been described under several names: *"Bacillus asiaticus"* and *"Bacillus asiaticus"* biovar "mobilis" (Castellani, 1912, cited by Ewing and Fife, 1968), *"Bacterium cadaveria"* (Gale and Epps, 1943), biotype 32011 of the genus *"Aerobacter"* (Stuart et al., 1943), *"Enterobacter alvei"* (Sakazaki, 1961), *Enterobacter aerogenes* subsp. *"hafniae"* (Ewing, 1963), and *"Enterobacter hafniae"* (Ewing and Fife, 1968). However, *Hafnia alvei* (Møller, 1954) is the only correct name for these organisms. Møller (1954) found a new group of organisms, in which a supposedly authentic strain of *"Bacillus paratyphi-alvei"* of Bahr (1919) was included. He proposed the name *Hafnia alvei* for this group of bacteria because he considered that Bahr's strain ought to be regarded as the type strain of this group. Sakazaki (1961) suggested the new combination *"Enterobacter alvei"* for *H. alvei*, because of its biochemical similarity to *Enterobacter*. Ewing and Fife (1968) pointed out that Bahr's strain, which had been designated as the type strain of *H. alvei* by Møller (1954), was not an authentic strain of this species, since biochemical reactions of the strain were not the same as those described by Bahr (1919). They considered therefore that the specific epithet *"alvei"* was illegitimate, and proposed the name *"Enterobacter hafniae"* for *H. alvei*. However, there is no doubt that Bahr's strain studied by Møller (1954) was a new bacterium at that time. In addition, numerical taxonomy studies by Johnson et al. (1975) and Gavini et al. (1976a) indicated that *Hafnia* strains retained a position separate from *Enterobacter*. In DNA–DNA hybridization studies, Steigerwalt et al. (1976) reported 11–26% relatedness of *H. alvei* with *Enterobacter* and *Klebsiella*. These data appear to justify the status of the *Hafnia* as a separate genus rather than including it in the genus *Enterobacter*.

Although only a single species, *Hafnia alvei*, has been designated, Steigerwalt et al. (1976) indicated in DNA reassociation studies that there were two genomospecies that were about 50% interrelated within the genus *Hafnia*. Overall phenotypic profiles may serve to separate the second subspecies from the named species *H. alvei*; however, it has not been named. Farmer et al. (1985a) referred to this second genomospecies as *Hafnia alvei* biogroup 1.

Priest et al. (1973) proposed that *Obesumbacterium proteus* Shimwell 1964, a common brewery contaminant, should be placed in the genus *Hafnia* as *"H. protea"*. They described two groups in this species by numerical analysis of phenotypic characteristics. Brenner (1981) determined DNA relatedness in both groups and found that one group appears to be a biovar of *H. alvei*, whereas the other is a new species that does not belong to the genus *Hafnia*.

Another group resembling *H. alvei*, *Yokenella regensburgei*, was first considered as a species of the genus *Hafnia*. Kosako et al. (1984), who studied the phenotypic and genotypic characteristics of both groups, confirmed that the former was distinct from members of the *Hafnia* and suggested the name *Yokenella regensburgei* for this group.

Acknowledgments

Riichi Sakazaki, who died in 2002, spent more than forty years at the Nippon Institute of Biological Sciences. His illustrious career focused on the classification and epidemiology of human and fish pathogens in Japan, particularly pathogenic bacteria in the families *Vibrionaceae* and *Enterobacteriaceae*. His contributions to Japanese bacteriology paralleled those of Drs. Edwards and Ewing in the U.S. He discovered and described many species in the genera *Enterobacter*, *Edwardsiella*, and *Vibrio*, and he developed serotyping schemes for *V. cholerae* and *V. parahaemolyticus*, as well as for several important species of *Enterobacteriaceae*. His research was always careful and comprehensive. He was one of the first Japanese scientists to publish in English language journals, thereby making his impressive accomplishments available to the world. He was recognized as one of the world's foremost experts on the genus *Vibrio* and on many genera in *Enterobacteriaceae*. He was a member of the WHO Subcommittees on the Taxonomy of *Vibrionaceae* and on the Taxonomy of *Enterobacteriaceae* for more than thirty years. He authored chapters on these organisms in three editions of *Bergey's Manual*. He was a good friend to the Bergey's Trust, as well as to all who knew him.

TABLE BXII.γ.227. Differential characteristics of the genus *Hafnia* and biochemically related genera[a]

Characteristic	*Hafnia*	*Enterobacter*	*Escherichia coli*	*Escherichia* strain STEC 0157[b]	*Salmonella*	*Serratia*	*Yokenella*
H_2S production	−	−	−	−	+	−	−
Indole production	−	−	+	+	−	D	−
β-Galactosidase	D	+	+	+	D	+	+
β-Glucuronidase	−	−	+	−	D	−	−
L-Pyrrolidonyl aminopeptidase	−	+	−	−	−	+	+
Sorbitol fermentation	−	D	+	−	+	D	−
Hafnia specific bacteriophage lysis[c]	+	−	−	−	−	−	−
Mol% G + C of DNA	48–49	52–60	48–52		50–53	52–60	58–59

[a]Symbols: +, 90–100% of strains are positive; −, 90–100% of strains are negative; D, different reactions given by different species or biogroups.

[b]Shiga toxin-producing *Escherichia coli* O157:H7.

[c]Data from Guinée and Valkenburg (1968).

Differentiation of the species of the genus *Hafnia*

Table BXII.γ.228 presents the biochemical characteristics of *Hafnia* species, which are also useful for differentiation between *H. alvei* and unnamed genospecies (genomospecies 2).

List of species of the genus Hafnia

1. **Hafnia alvei** Møller 1954, 272[AL]
al' ve.i. L. n *alveus* a beehive; L. gen. n. *alvei* of a beehive.

The morphology is as given for the genus. Motility is most pronounced at 30°C. Nonmotile strains may be encountered occasionally.

Grows readily on ordinary media. Colonies on nutrient agar are generally 2–4 mm in diameter, smooth, moist, translucent, and gray, with a shiny surface and entire edge. Rare strains may produce mucoid colonies. Physiological and biochemical characteristics are presented in Table BXII.γ.228.

Found in the feces of humans and other animals, including birds. Also found in sewage, soil, water, and dairy products. It may be isolated in association with some pathological processes in patients with underlying illness. Some strains may cause diarrheal diseases.

The mol% G + C of the DNA is: 48.0–48.7 (T_m).
Type strain: ATCC 13337, DSM 30163, NCTC 8106.
GenBank accession number (16S rRNA): M59155.

TABLE BXII.γ.228. Characteristics of *Hafnia alvei* and genomospecies 2[a]

Characteristic	*H. alvei*	Genomospecies 2
Indole production	–	–
Voges–Proskauer test (25°C)	+	+
Voges–Proskauer test (35°C)	d	d
Citrate (Simmons) (25°C)	d	–
Citrate (Simmons) (35°C)	–	–
Lysine decarboxylase	+	+
Arginine dihydrolase	–	–
Ornithine decarboxylase	+	d
H_2S production (Kligler)	–	–
Phenylalanine deaminase	–	–
Urease (Christensen)	–	–
β-Galactosidase	+	d
β-Glucuronidase	–	–
Malonate utilization	d	d
L-Pyrrolidonyl aminopeptidase	–	–
Gelatinase	–	–
Lipase (Tween 80)	–	–
Deoxyribonuclease	–	–
Growth in KCN medium	+	–
Esculin hydrolysis	–	–
Gas from D-glucose	+	–
Acid from carbohydrates:		
L-Arabinose, maltose, L-rhamnose, D-xylose	+	–
Lactose, melibiose, raffinose, sucrose[b], adonitol, D-arabitol, dulcitol, *myo*-inositol, D-sorbitol, α-methyl-D-glucoside	–	–
Cellobiose	d	–
D-Mannitol	+	d
Trehalose	+	d
Salicin	d	d

[a]For symbols see standard definitions.
[b]Late positive reactions are given by approximately 50% of the strains of *H. alvei*.

Genus XVI. **Klebsiella** *Trevisan 1885, 105[AL] emend. Drancourt, Bollet, Carta and Rousselier 2001, 930*

Patrick A.D. Grimont and Francine Grimont

Kleb.si.el' la. M.L. dim. ending *-ella;* M.L. fem. n. *Klebsiella* named after Edwin Klebs (1834–1913), a German bacteriologist.

Straight rods, 0.3–1.0 × 0.6–6.0 µm, arranged singly, in pairs or short chains; often surrounded by a **capsule**. Conform to the general definition of the family *Enterobacteriaceae*. Gram negative. **Nonmotile** (except *K. mobilis*). Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow on meat extract media (except *K. granulomatis*, which has not been cultured), producing more or less dome-shaped, glistening colonies of varying degrees of stickiness depending on the strain and the composition of the medium. **Oxidase negative.** Glucose is fermented with the production of acid and gas (more CO_2 is produced than H_2), but anaerogenic strains occur. Most strains produce 2,3-butanediol as a major end product of glucose fermentation. The **Voges–Proskauer test is usually positive**. Lactic, acetic, and formic acids are formed in smaller amounts and ethanol in larger amounts than in a mixed acid fermentation. All strains utilize L-arabinose, D-arabitol, D-cellobiose, citrate, D-fructose, D-galactose, D-glucose, 2-ketogluconate, maltose, D-mannitol, D-melibiose, D-raffinose, D-trehalose, and D-xylose as sole carbon sources. With the exception of some *K. pneumoniae* subsp. *ozaenae* strains, all *Klebsiella* strains **utilize *myo*-inositol, L-rhamnose, and sucrose as sole carbon sources**. With the exception of some *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis* strains, all strains utilize lactose and D-sorbitol as sole carbon sources. No strain utilizes betaine, caprate, caprylate, glutarate, itaconate, 3-phenylpropionate, and propionate. H_2S is not produced, β-glucuronides are not hydrolyzed, and L-tryptophan and L-histidine are not deaminated. Ornithine is not decarboxylated by klebsiellae strains except *K. mobilis*, *K. ornithinolytica*, and rare strains of *K. pneumoniae*. Most strains hydrolyze urea and β-galactosides. Some strains fix nitrogen. Occur in intestinal

contents, clinical specimens from humans and animals (e.g., horses, swine, monkeys), soil, water, or on plants.

The mol% G + C of the DNA is: 53–58.

Type species: **Klebsiella pneumoniae** (Schroeter 1886) Trevisan 1887, 94 (*Hyalococcus pneumoniae* Schroeter 1886, 1952.)

Further descriptive information

Phylogenetic treatment The agent of donovanosis, *Calymmatobacterium granulomatis*, was found to be so closely related to *Klebsiella pneumoniae* by *rrs* (16S rRNA gene) sequence comparison that the species was transferred to the genus *Klebsiella* as *Klebsiella granulomatis* (Carter et al., 1999; Kharsany et al., 1999). However, it is not known whether *K. granulomatis* is a species distinct from *K. pneumoniae*.

The phylogenetic position of klebsiellae has been addressed by *rrs*, *rpoB* (Drancourt et al., 2001), *gyrA*, and *parC* (Brisse and Verhoef, 2001) sequence comparison.

The genus *Klebsiella* was found to be polyphyletic by Drancourt et al. (2001). Cluster I contained *Klebsiella pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *rhinoscleromatis*, and *K. granulomatis*. Cluster II contained *K. planticola*, *K. ornithinolytica*, and *K. terrigena*. Cluster III contained *K. oxytoca*. As a consequence, cluster II was proposed to constitute a new genus, *Raoultella* (Drancourt et al., 2001). The position of *Klebsiella mobilis* (*Enterobacter aerogenes*) was very close to cluster II, although the proposal by Drancourt et al. (2001) did not include that species.

A somewhat different structure was found by Brisse and Verhoef (2001), who uncovered two groups. The first group contained *K. pneumoniae* with its three subspecies, and the second group contained *K. oxytoca*, *K. planticola*, *K. ornithinolytica*, *K. terrigena*, and *K. mobilis*. Furthermore, three clusters (KpI to KpIII) were evidenced in *K. pneumoniae*, which did not correlate with the named subspecies. These clusters may have different habitats and different physiological properties (D-adonitol fermentation, for example). *K. oxytoca* was composed of two clusters, the significance of which is unknown.

Cell morphology and cell wall composition The outermost layer of *Klebsiella* bacteria consists of a large polysaccharide capsule, a characteristic that distinguishes members of this genus from most other bacteria in the family (*Escherichia coli* strains with a heat-stable K antigen may form similar capsules). The cell wall is structured similarly to other *Enterobacteriaceae*. Above the cytoplasmic membrane is the peptidoglycan layer and the outer membrane containing lipopolysaccharide (LPS). In addition, *Klebsiella* strains may possess fimbriae (pili) (Duguid, 1959).

The production of the large capsules gives rise to large mucoid colonies of a viscid consistency. The capsular material also diffuses freely into the surrounding liquid medium as extracellular capsular material.

Nutrition and growth conditions Best results with biochemical tests are obtained when *Klebsiella* cultures are incubated at 30–35°C. *Klebsiella* strains (except *K. granulomatis*) grow readily on all kinds of media commonly used to isolate *Enterobacteriaceae*, such as nutrient agar, tryptic casein soy agar, bromocresol purple lactose agar, Drigalski agar, MacConkey agar, eosin-methylene blue (EBM) agar, and bromothymol blue (BTB) agar. *K. pneumoniae* and *K. oxytoca* colonies are lactose positive, more or less dome-shaped, 3–4 mm in diameter after overnight incubation at 37°C or 30°C, with a mucoid aspect and sometimes stickiness depending on the strain and the composition of the medium. *K. planticola*, *K. terrigena*, *K. ornithinolytica*, and *K. mobilis* (*Enterobacter aerogenes*) colonies are also lactose positive, 1.5–2.5 mm in diameter, dome-shaped, with a weakly mucoid aspect. *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *rhinoscleromatis*, and occasionally *K. pneumoniae* K1 strains grow more slowly on the same media, yielding voluminous, rounded, very mucoid, translucent, and confluent colonies in 48 h at 30°C or 37°C (Ørskov, 1981; Richard, 1982). Similar colonies, indistinguishable from those of *Klebsiella*, may be formed by other genera of the *Enterobacteriaceae*, particularly *E. coli* mucoid varieties with capsular K antigens.

The use of a carbohydrate-rich medium, such as bromothymol blue lactose agar or Hajna or Worfel–Ferguson medium, produces a better formed capsule than a carbohydrate-poor medium.

Almost all *Klebsiella* strains grow in minimal medium with ammonium ions or nitrate as sole nitrogen source and a carbon source, without growth factor requirement. Some *K. pneumoniae* K1 isolates require arginine or adenine or both as growth factors. *K. pneumoniae* subsp. *rhinoscleromatis* requires arginine and uracil but growth factor requirements of *K. pneumoniae* subsp. *ozaenae* are not fully determined (leucine, cysteine, and methionine are stimulatory). Ornithine can replace arginine for these requirements (Grimont et al., 1991).

K. granulomatis has not been grown axenically in artificial media. Cultures have been achieved *in vivo* in the yolk sac of developing chick embryo and in the developing chick embryo brain. Growth can be obtained in cell cultures, fresh mononuclear cells (Kharsany et al., 1997) or in Hep-2 human epithelial cell line (Carter et al., 1997).

Metabolism The characteristics useful for the identification of *Klebsiella* species are given in Table BXII.γ.229.

The Voges–Proskauer (VP) test is usually positive in *Klebsiella*, meaning that acetoin and 2,3-butanediol are produced during glucose fermentation. Some strains of *K. pneumoniae* subsp. *rhinoscleromatis*, do not form acetoin and 2,3-butanediol. In some strains, 2,3-butanediol is utilized and acetoin will disappear before the VP reaction is tested. Richard's modification of the VP test (Le Minor and Richard, 1993) most often gives positive results with *Klebsiella* strains (except *K. pneumoniae* subsp. *ozaenae* and subsp. *rhinoscleromatis*).

Carbon source utilization tests are conveniently obtained with Biotype-100 strips (BioMérieux, La Balme-les-Grottes, France) used with a minimal medium (Biotype Medium 1) containing 16 growth factors. *K. pneumoniae* subsp. *rhinoscleromatis* and some strains of *K. pneumoniae* subsp. *ozaenae* may need Biotype medium 2, which contains all known growth factors for enteric bacteria. The strips are examined for visual growth after 2 and 4 d (Grimont et al., 1991). Almost all *Klebsiella* strains utilize the following compounds as sole carbon and energy sources (provided growth factor requirements of some strains are met): *N*-acetyl-D-glucosamine, L-alanine, L-arabinose, D-arabitol, L-aspartate, D-cellobiose, citrate, D-fructose, L-fucose, fumarate, D-galactose, gentiobiose, D-gluconate, D-glucosamine, D-glucose, DL-glycerate, glycerol, *myo*-inositol, 2-ketogluconate, DL-lactate, lactose, D-malate, L-malate, maltose, maltotriose, D-mannitol, D-mannose, D-melibiose, 1-*O*-methyl-β-glucoside, L-proline, D-raffinose, D-ribose, L-serine, D-trehalose, and D-xylose. Esculin is hydrolyzed. H_2S, β-glucuronidase, phenylalanine deaminase, and tryptophan deaminase are not produced; DNA and tributyrin are not hydrolyzed. Almost no strain of *Klebsiella* can utilize the following com-

TABLE BXII.γ.229. Differentiation characteristics of the species and subspecies of the genus *Klebsiella*[a]

Characteristics	1a. *K. pneumoniae* subsp. *pneumoniae*	1b. *K. pneumoniae* subsp. *ozaenae*	1c. *K. pneumoniae* subsp. *rhinoscleromatis*	3. *K. mobilis*	4. *K. oxytoca*	5. *K. planticola*	6. *K. terrigena*	Species *incertae sedis* *K. ornithinolytica*
Motility	−	−	−	+	−	−	−	−
Growth at:								
5°C	−	−	−	+	−	+	+	+
41°C	+	+	+	+	+	d	−	+
44.5°C	+	nd	nd	nd	d	−	−	nd
Urea hydrolyzed	+	d	−	−	+	+	+	+
Pectate hydrolyzed	−	−	−	−	+	−	−	−
ONPG test	+	+	−	+	+	+	+	+
Indol produced	−	−	−	−	+	d	−	+
Voges–Proskauer test	+	−	−	+	+	+	+	+
Malonate test	+	−	+	+	+	+	+	+
Lysine decarboxylated	+	d	−	+	+	+	+	+
Ornithine decarboxylated	−	−	−	+	−	−	−	+
Glucose dehydrogenase:								
Without added pyrroloquinoline quinone	+	−	−	+	−	−	−	nd
With added pyrroloquinoline quinone	+	−	−	+	+	+	+	nd
Gluconate dehydrogenase	+	−	−	+	−	+	+	nd
Utilization of:								
Adonitol	d	+	(+)	+	+	+	+	+
D-Alanine	+	+	−	+	+	+	+	+
L-Arabitol	−	−	−	−	d	−	−	−
Benzoate	d	−	−	+	d	+	d	+
m-Coumarate	d	d	−	−	+	+	−	+
Dulcitol	d	−	−	d	d	d	−	d
i-Erythritol	−	−	−	−	−	(d)	−	d
D-Galacturonate	+	+	−	+	+	+	+	+
Gentisate	−	−	−	+	+	−	d	−
D-Glucuronate	+	+	−	+	+	+	+	+
Histamin	−	−	−	(+)	−	+	d	d
3-Hydroxybenzoate	−	−	−	+	+	−	(+)	−
4-Hydroxybenzoate	+	−	−	d	+	+	+	+
5-Ketogluconate	d	−	−	d	+	+	+	+
Lactulose	+	d	−	(+)	+	+	(+)	+
Malonate	d	−	d	d	(d)	(d)	−	d
Maltitol	+	+	−	+	+	+	+	+
D-Melezitose	−	−	−	−	d	−	+	−
1-*O*-Methyl-β-galactoside	+	+	−	+	+	+	+	+
3-*O*-Methyl-D-glucose	−	−	−	−	−	+	+	+
1-*O*-Methyl-α-D-glucoside	d	+	−	+	+	+	+	+
Mucate	+	d	−	+	+	+	+	(+)
Palatinose	+	+	−	+	+	+	+	+
Phenylacetate	d	d	−	+	+	+	+	+
Protocatechuate	+	d	−	+	+	+	+	+
Putrescine	d	d	−	d	(+)	+	(+)	+
Quinate	+	d	−	+	+	+	+	+
L-Rhamnose	+	d	+	+	+	+	+	+
D-Saccharate	+	d	d	+	+	+	+	(+)
D-Sorbitol	+	d	d	+	+	+	+	+
L-Sorbose	d	d	−	−	+	+	+	+
Sucrose	+	d	+	+	+	+	+	+
D-Tagatose	d	−	−	d	d	d	−	d
D-Tartrate	d	−	−	−	−	(d)	−	d
Tricarballylate	d	−	−	+	+	−	+	−
Trigonelline	−	−	−	d	−	−	−	−
Tryptamine	−	−	−	d	−	−	−	−
D-Turanose	d	d	−	d	d	(d)	−	d
L-Tyrosine	−	−	−	d	−	−	−	−
D-Xylitol	d	−	−	d	(d)	d	−	d

[a]Symbols: +, 95–100% strains positive in 1–2 d (utilization tests) or in 1 d (other tests); (+), 95–100% strains positive in 1–4 d; −, 95–100% strains negative in 4 d; d, different reactions; nd, not determined.

pounds as sole carbon and energy sources after 4-d incubation: betaine, caprate, caprylate, glutarate, itaconate, 3-phenylpropionate, and propionate. It is obvious from Table BXII.γ.229 that *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis* are biotypes of *K. pneumoniae* with less nutritional versatility. There is no substrate utilized by subsp. *ozaenae* and subsp. *rhinoscleromatis* that is not utilized by subspecies subsp. *pneumoniae*. For this reason, it is necessary to check the identification

of these less active subspecies by capsular typing. Carbon source utilization tests are essential for the precise identification of *Klebsiella* species (Grimont et al., 1991). In Biotype-100 strips, *K. oxytoca* often produces a soluble yellow compound in all cupules showing some growth, and this may be analogous to the pigment produced by this species on ferric gluconate (Ørskov, 1984b). Growth on gentisate and 3-hydroxybenzoate never occurs with *K. pneumoniae* and *K. planticola*. Growth on histamine never occurs with *K. pneumoniae* and *K. oxytoca*. Growth on D-sorbose is always negative with *K. mobilis* (*E. aerogenes*) and growth on tricarballylate is always negative with *K. planticola*. Only *K. terrigena* and some strains of *K. oxytoca* can grow on D-melezitose, and only *K. planticola* and *K. terrigena* can grow on 3-*O*-methyl-D-glucose. These carbon sources are often sufficient for the identification of all *Klebsiella* species (Grimont et al., 1991).

Oxidation of glucose to gluconate (mediated by glucose dehydrogenase) in the absence of added pyrroloquinoline quinone is a distinctive property of *K. pneumoniae* subsp. *pneumoniae* and *K. mobilis* (Bouvet et al., 1989). The other species require the addition of cofactor pyrroloquinoline quinone to express glucose dehydrogenase activity. Gluconate is oxidized into 2-ketogluconate (by a gluconate dehydrogenase) by *K. pneumoniae* subsp. *pneumoniae*, *K. planticola*, *K. terrigena*, and *K. mobilis*, but not by *K. oxytoca*, *K. pneumoniae* subsp. *ozaenae*, and *K. pneumoniae* subsp. *rhinoscleromatis*. *Klebsiella* species do not oxidize 2-ketogluconate to 2,5-ketogluconate (Bouvet et al., 1989).

K. pneumoniae subsp. *pneumoniae* can grow fermentatively on glycerol thanks to a glycerol dehydrogenase type I (induced by glycerol and dihydroxyacetone) and 1,3-propanediol dehydrogenase, which are typical enzymes of the anaerobic glycerol dissimilation pathway (Bouvet et al., 1995a). Other species of *Klebsiella* cannot grow fermentatively on glycerol and possess a glycerol dehydrogenase, but no 1,3-propanediol dehydrogenase (Bouvet et al., 1995a).

Some *K. oxytoca* and *K. planticola* strains are capable of fixing nitrogen and are classified as associative nitrogen fixers (Ladha et al., 1983). Nitrogen fixation is catalyzed by the enzyme nitrogenase in the absence of combined nitrogen, and under anaerobic conditions. Nitrogen fixation in the facultative anaerobe *K. pneumoniae* occurs only under anaerobic or microaerobic conditions. Low O_2 concentrations enhance nitrogenase synthesis; higher O_2 concentrations inhibit both synthesis and activity (Hill et al., 1984).

Genetic recombination has been reported in *Klebsiella* (Matsumoto and Tazaki, 1970), and *K. pneumoniae* has been used by several workers for detailed genetic analysis of the genes involved in N_2 fixation (*nif* genes). These genes are clustered near the *his* region on the chromosome but can be mobilized and transferred to other organisms. The genetics and regulation of nitrogen fixation in *Klebsiella* have been reviewed by Gussin et al. (1986).

Klebsiella strains may harbor a *lac* plasmid giving a stronger lactose positive phenotype (Reeve and Braithwaite, 1973).

Klebsiella strains may be lysogenic, but phages used by some workers for phage typing have been isolated from stools or sewage (Slopek et al., 1967; Slopek, 1978). Many *Klebsiella* strains produce bacteriocin (klebecin) and typing sets of such producers can be selected (Slopek and Maresz-Babczyszyn, 1967; Edmondson and Cooke, 1979).

Antigenic structure The outer membrane contains the lipopolysaccharide (LPS) that forms the O-antigen. The oligosaccharide repeating units of the O-antigen consists of only a few carbohydrate components. Some of the O-antigens are homopolysaccharides such as galactans or mannans. There are only 12 different O-antigens. O-antigen determination is difficult because it is hampered by the stable K-antigens (Ørskov and Ørskov, 1984b).

Klebsiellae are enveloped by a polysaccharide capsule of considerable thickness. This constitutes the capsular or K-antigen. The capsular polysaccharide also diffuses freely into the liquid medium as extracellular capsular material. The *Klebsiella* capsular polysaccharides are acidic and composed of repeating units. Only a few carbohydrate constituents are found. Most K-antigens contain charged monosaccharide constituents such as glucuronic and hexoses, occasionally 6-deoxyhexoses. Noncarbohydrate constituents such as formyl, acetyl, or pyruvate groups are found. Following the pioneer work of Julianelle (1926), who determined the first three capsular types, a total of 82 K-antigens (K:1 to K:82) were described, although K:73, K:75, K:76, and K:78 were invalidated. There are thus only 78 available types. Cross-reactions among established K-antigens are numerous. K-antigen determination is traditionally by the capsular swelling reaction. When bacteria and the corresponding serum are mixed on a glass slide and observed under the microscope, the capsule becomes highly refractile and easily visible. Other methods include slide or tube agglutination, indirect immunofluorescence, and countercurrent immunoelectrophoresis (Ørskov and Ørskov, 1984b).

Some K-antigens are correlated with pathogenicity. *K. pneumoniae* subsp. *rhinoscleromatis* strains have capsular antigen K:3, whereas *K. pneumoniae* subsp. *ozaenae* strains are mostly K:4 and occasionally K:3 or K:5. *K. pneumoniae* subsp. *pneumoniae* strains recovered from respiratory tract infections have generally antigens K:1 to K:6, whereas strains form other nosocomial infections (e.g., urinary tract) have often antigens K:2, K:21, K:55, K:10, or K:24. Other K-types (17, 25, 22, 43, 1, 3, 33) can also be encountered (Le Minor and Richard, 1993). Strains found in the sperm of stallions and that cause metritis and abortion in mares contaminated with such sperm are often K:1, K:2, K:7, or K:30. Nosocomial *K. oxytoca* strains have been found with K:26, K:68, K:43, and less frequently K:26, K:21, K:18, or K:47. *K. mobilis* (*E. aerogenes*) strains most of the time can show capsular swelling with *Klebsiella* antisera. K-types associated with this species are often K:68 or K:26 and occasionally K:42, K:59, K:11, or K:4 (Le Minor and Richard, 1993).

Pathogenicity The genus *Klebsiella* is ubiquitous. Strains of this genus are found in surface water, sewage, soil, and on plants. They also colonize the mucosal surfaces of humans, horses, and swine (Podschun and Ullmann, 1998). In humans, *K. pneumoniae* is present as a commensal in the nasopharynx (detection rate, 1–6%) and in the intestinal tract (detection rate, 5–38%). In hospitals, colonization rates increase with the length of stay (detection rates, 77% in the stool, 19% in the pharynx, and 42% on the hands of patients) and hospital personnel can carry *K. pneumoniae* (Podschun and Ullmann, 1998). The high rate of colonization in patients is associated with the use of antibiotics.

The genus *Klebsiella* can be associated with different sorts of infections. *Klebsiella pneumoniae* is a cause of community-acquired bacterial pneumonia (Friedländer's pneumonia), occurring particularly in chronic alcoholics, and showing characteristic radio-

graphic abnormalities. The fatality rate is high if untreated (Podschun and Ullmann, 1998).

K. pneumoniae subsp. *rhinoscleromatis* is the causative agent of rhinoscleroma, a chronic infection that can involve the nasal cavity (most often) or the upper airways (pharynx, larynx, trachea). Typically, a granulomatous destructive and disfiguring process results in airway obstruction. Ultimately, extensive fibrosis and scarring occur. Histological features include mixed diffuse infiltrate of the upper and lower dermis with numerous plasmocytes, lymphocytes, neutrophils, and characteristic large macrophages with foamy cytoplasm (Mikulicz cells) and that contain *Klebsiella*.

K. pneumoniae subsp. *ozaenae* has been implicated in ozena, a chronic atrophic rhinitis giving off a very bad smell. However, the pathogenic role of the bacterium in this syndrome is less clear.

Klebsiella granulomatis (formerly *Calymmatobacterium granulomatis*) is the presumed causative agent of granuloma inguinale (donovanosis), a genital ulceration that is sexually transmissible. Histological features include dense dermal infiltrate of plasmocytes, neutrophils, and large macrophages with vacuolated cytoplasms that contain intracellular bacilli (Donovan bodies). The similarity of donovanosis and rhinoscleroma histological lesions has been pointed out (Carter et al., 1999).

Klebsiella spp. (with the exception of *K. pneumoniae* subsp. *rhinoscleromatis*, *K. pneumoniae* subsp. *ozaenae*, and *K. granulomatis*) are mostly considered nosocomial pathogens. *Klebsiella pneumoniae* and *K. mobilis* (*Enterobacter aerogenes*) are most frequently involved, although *K. oxytoca* and *K. planticola*, and rarely *K. terrigena*, can be found.

The urinary tract is the most common site of infection, and *K. pneumoniae* accounts for 6–17% of all nosocomial urinary tract infections (Podschun and Ullmann, 1998). *K. pneumoniae* is also a frequent cause of bacteremia. In premature infants, *K. pneumoniae* is often involved in neonatal sepsis.

The principal reservoir of *K. pneumoniae* in the hospital is the gastrointestinal tract of patients. The principal vectors are the hands of personnel. Occasional sources are contaminated medical equipment and blood products (Podschun and Ullmann, 1998).

Renewed interest in *Klebsiella* infections stemmed from the spread of multiresistant strains. *K. pneumoniae* is naturally susceptible to aminoglycosides. However, plasmids mediating aminoside-modifying enzymes have spread in the 1970s. In a French survey, 78% of *Klebsiella* isolates were susceptible to gentamicin, tobramycin, netilmycin, and amikacin. Different phenotypes of aminoside resistance were observed, involving resistance to gentamicin, tobramycin, netilmycin, and/or amikacin (Lemozy et al., 1985). The most frequent aminoside inactivating enzymes found produced by *Klebsiella* isolates were aminoglycoside-3′-*N*-acetyltransferases and aminoglycoside -2′′-*O*-nucleotidyltransferase (Lemozy et al., 1985).

Strains of *K. pneumoniae* and *K. oxytoca* that have not acquired any resistance determinant are naturally resistant to aminopenicillins (ampicillin) and carboxypenicillins (carbenicillin) and susceptible to other β-lactam antibiotics. This is due to the production of a chromosomal penicillinase, which is inhibited by clavulanic acid. A small zone of inhibition around 100–mg carbenicillin disks is typical of this phenotype (Jarlier, 1985). Acquired resistance arose from the production of a plasmid-determined penicillinase. The strains showed a higher resistance to carbenicillin (no zone around carbenicillin disks), and resistance to ureidopenicillins, cefalothin, cefamandole, and cefuroxime. Inactivation of amoxycillin was not blocked by clavulanic acid. However, these strains remained susceptible to third-generation cephalosporins (e.g., cefotaxime) (Jarlier, 1985). Since 1982, strains producing extended spectrum β-lactamases (ESBL) encoded by a plasmid have spread. *K. pneumoniae* strains resistant to ceftazidime were commonly producing a SHV-5 type β-lactamase in Europe and a TEM-10 or TEM-12 type in the United States. These strains account for 5% (USA) to 16% (France) of the *K. pneumoniae* tested (Podschun and Ullmann, 1998). These ESBL-producing strains have been susceptible to carbapenems (imipenem, meropenem). However, ESBL-producing *K. pneumoniae* resistant to imipenem have been isolated. They produce a β-lactamase of the AmpC type (Podschun and Ullmann, 1998). *Klebsiella mobilis* (*Enterobacter aerogenes*) was rarely encountered as a nosocomial pathogen until strains carried a plasmid encoding an ESBL. In France and Belgium, β-lactamases of type SHV-4, TEM-24, and TEM-3 were characterized in *K. mobilis*. It was shown that TEM-24 producing isolates found in several epidemics in different countries constituted mostly a single clone (Galdbart et al., 2000; De Gheldre et al., 2001; Mammeri et al., 2001).

Pathogenesis *Klebsiella* species are surrounded by a hydrophilic polysaccharide capsule, which is the first virulence factor described in klebsiellae (Toenniessen, 1914; Cryz et al., 1984). Simoons-Smit et al. (1984) showed that strains with K1, K2, K4, and K5 capsular antigens were more virulent for mice (skin model) than strains with K6 and K above 6. The loss of K antigen by K^+ strains resulted in the reduced virulence of their K^- variants. This reduced virulence may be explained by a higher degree of phagocytosis as measured by chemiluminescence response of human polymorphonuclear leukocytes (PMNLs) and by enhanced killing by either human PMNLs or human serum or both (Simoons-Smit et al., 1986). Other studies (mouse peritonitis model) found strains with antigens K1 and K2 more virulent than strains with other K antigens (Podschun and Ullmann, 1998). The K antigen plays a crucial role in protecting the bacterium from opsonophagocytosis in the absence of specific antibodies (Williams et al., 1983, 1986b; Simoons-Smit et al., 1986). The antiphagocytic function consists of inhibiting the activation or uptake of complement components, especially C3b. In addition, the K antigen inhibits the differentiation and functional capacity of macrophages *in vitro*. The degree of virulence conferred by a particular K antigen might be connected to the mannose content of the capsular polysaccharide (Podschun and Ullmann, 1998). K2 serotype is among the most common K-type isolated from patients with urinary tract infection, pneumonia, or bacteremia. K2 is the predominant serotype of human isolates worldwide, whereas K2 strains are very rarely encountered in the environment (Podschun and Ullmann, 1998).

Strains of *K. pneumoniae*, *K. oxytoca*, *K. planticola*, and *K. terrigena* may produce thick, channeled (type-1) fimbriae associated with mannose-sensitive hemagglutination (MS-HA). *K. mobilis* (*E. aerogenes*) produces antigenically similar fimbriae. Type 1 fimbriae mediate the attachment of *K. pneumoniae* to uroepithelial cells (Williams and Tomas, 1990) and to ciliated tracheal cells *in vitro* (Fader et al., 1988).

Strains of *K. pneumoniae*, *K. oxytoca*, *K. planticola*, and *K. terrigena* may also produce thin, nonchanneled (type-3) fimbriae associated with mannose-resistant hemagglutinins of the *Klebsiella* type (MR/K-HA). These fimbriae are efficient in promoting adherence to the roots of various grasses and cereals (Haahtela and

Korhonen, 1985). They are capable of binding to endothelial cells, respiratory tract epithelial cells, and uroepithelial cells. In the kidney, they mediate bacterial adhesion to tubular basement membranes, Bowman's capsules, and renal vessels (Podschun and Ullmann, 1998). Three new putative colonization factors have also been described (Podschun and Ullmann, 1998).

The most important role of the O antigen is to protect *K. pneumoniae* from complement-mediated killing (Williams and Tomas, 1990). For this protection, O antigen chain length seems to be important (McCallum et al., 1989).

Iron is essential for bacterial growth. Brewer et al. (1982) observed that virulence was enhanced by hyperferremi. Since in the human body iron is complexed to carrier molecules such as transferrin (in the serum) or lactoferrin (in milk and other secretions), or sequestered within cells (in heme proteins), potentially pathogenic *Enterobacteriaceae* produce high-affinity systems (siderophores) to solubilize and import the required iron. The iron-chelating compounds produced are mostly of two sorts, phenolates (e.g., enterochelin) and hydroxamates (aerobactin) (Payne, 1988). Almost all strains of *Klebsiella* produce enterochelin whereas only a few produce aerobactin (Williams et al., 1987). Nassif and Sansonetti (1986) correlated the virulence of *K. pneumoniae* serotypes K1 or K2 with the presence of a 180-kb plasmid encoding the hydroxamate siderophore aerobactin. Aerobactin is an essential factor of pathogenicity and the 180-kb plasmid carries additional genes encoding other virulence factors (Nassif and Sansonetti, 1986). Some strains of *K. pneumoniae* express a ferric aerobactin uptake system without making the chelator itself. This may confer a selective advantage in mixed infections in competition with other aerobactin-producing bacteria (Williams and Tomas, 1990).

The production of cytotoxins, enterotoxins, and hemolysins have been sporadically described (Podschun and Ullmann, 1998).

Ecology Klebsiellae have been recovered from aquatic environments receiving industrial wastewaters, plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugar cane wastes, living trees, plants, and plant by-products. They are commonly associated with wood, saw dust, and waters receiving industrial effluents from pulp and paper mills and textile finishing plants. Isolates have been described in forest environments, degrading kraft-lignin, tannic acid, pine bark, and condensed tannin, or from living or decaying wood and bark or composted wood.

Klebsiella can frequently be isolated from the root surfaces of various plants. *K. pneumoniae*, *K. oxytoca*, or *K. planticola* are capable of fixing nitrogen and are classified as associative nitrogen fixers.

Strains from plants certainly need to be reidentified in the light of present taxonomic schemes. Strains of *K. pneumoniae sensu stricto* that are associated with plants differ from those associated with serious human infections. These environmental *K. pneumoniae* strains are most often able to utilize 5-ketogluconate as sole carbon source and never have capsular types K1 to K6. Strains involved in serious infection do not utilize 5-ketogluconate and may have capsular types K1 to K6 as well as other capsular types (Grimont et al., 1991).

Capsular types K1, K2, and K5 were major causes of epidemic metritis in mares in England, whereas type K7 was associated with sporadic, opportunistic genital infection. Outbreaks of metritis in mare were due to type K2 in the United States and in France. The stallion plays an important role in the transmission of *K. pneumoniae*. Type K7 was found on the preputial skin of stallions and may be part of the normal bacterial flora in this location. Thus it is important to type *Klebsiella* isolated in the genital tract of horses to detect a stallion carrying an epidemic strain among other stallions carrying less pathogenic *K. pneumoniae* (Grimont et al., 1991).

Klebsiella have been frequently associated with bovine mastitis, and causes serious infections in other animals including rhesus monkeys, guinea pigs, or muskrats. Epidemics of fatal generalized infections among captive squirrel monkeys (*Saimiri sciureus*) in French Guyana and lemurs in a French zoo were due to *K. pneumoniae* K5 and K2, respectively. Immunization of the monkeys with the corresponding capsular polysaccharide is efficient in stopping the epidemic (Grimont et al., 1991).

Enrichment and Isolation Procedures

The detection, isolation, and enumeration in clinical, industrial, and natural environments can be facilitated by using a selective medium.

On agar plates, although colonies develop overnight, the characteristic elevated and mucoid appearance is observed after incubation for 48 h.

The ability to utilize citrate (Cooke et al., 1979) or *myo*-inositol (Legakis et al., 1976) has been applied to the formulation of selective media. Resistance of *Klebsiella* spp. to methyl violet (Campbell and Roth, 1975), double violet (Campbell et al., 1976), potassium tellurite (Tomas et al., 1986), and carbenicillin (Thom, 1970) has been used in selective media.

Thom (1970) developed a medium based on the MacConkey agar in which lactose is replaced by inositol (1% w/v), with the addition of 100 μg of carbenicillin per ml. Bagley and Seidler (1978) devised a similar medium with only 50 μg/ml carbenicillin. On this medium, about 95% of pink-to-red colonies were verified to be *Klebsiella* spp., whereas only 1% of yellow background colonies were *Klebsiella*.

Since about 10% of *Klebsiella* strains are susceptible to 50 μg/ml of carbenicillin, the antibiotic was replaced in the above formula by tellurite (K_2TeO_3, 3 μg/ml) (Tomas et al., 1986), which is a strong inhibitor of phosphate transport in *E. coli*. Minimal inhibitory concentrations of K_2TeO_3 were 100 or 200 μg/ml for *K. pneumoniae* subsp. *pneumoniae*, *K. oxytoca*, *K. planticola*, and *K. terrigena*, 10 μg/ml for *K. pneumoniae* subsp. *ozaenae*, and 1–3 μg/ml for other *Enterobacteriaceae* (Tomas et al., 1986). In a field test, 77% of pink-to-red colonies on MacConkey–inositol–potassium tellurite agar were confirmed as *Klebsiella* spp.; however, the efficiency of plating was about 1% (Dutka et al., 1987).

Bruce et al. (1981) devised an agar medium combining Koser citrate and raffinose (carbon sources) and ornithine and low pH (for ornithine decarboxylase). On acidic Koser citrate agar with ornithine and raffinose, *Klebsiella* strains grow as yellow mucoid colonies. Other *Enterobacteriaceae* either do not grow, or produce small colorless, pink, red, or orange colonies.

For the isolation of *K. pneumoniae* and *K. oxytoca* from human feces, Van Kregten et al. (1984) have developed a medium based on the presence of two carbon sources, citrate and inositol, without inhibitor. The medium consists of Simmons citrate agar with 1% inositol. *Klebsiella* spp. appear as yellow, dome-shaped, often mucoid colonies, whereas *E. coli* appears as tiny, watery colonies. Apart from some *Enterobacter* strains, no other of bacteria grow on the medium.

Wong et al. (1985) devised a minimal medium in which the carbon source is lactose and the nitrogen source is potassium nitrate. Inhibitory compounds were deoxycholate, neutral red, and crystal violet. On this medium, *K. pneumoniae* and *K. oxytoca* grow as convex, and 1–2 mm in diameter, rather mucoid pink-to-red colonies, or larger, more watery pale red colonies with a dark red center. Non-klebsiellae either fail to grow or form colorless colonies.

Maintenance Procedures

Klebsiella strains can be easily maintained at room temperature in meat extract semisolid agar, at −80°C in a broth medium with 10–50% (v/v) glycerol, or freeze-dried. *K. granulomatis* will not remain viable when stored at 5° or 37°C. At 25°C, viability of egg yolk cultures is maintained for 8–10 d.

Differentiation of the genus *Klebsiella* from other genera

See Table BXII.γ.193 of the family *Enterobacteriaceae* for characteristics that can be used to distinguish *Klebsiella* from other genera of the family. No strain of *Klebsiella* other than *K. mobilis* is motile.

Classically, a Gram-negative capsulated isolate that is nonmotile, aerogenic, Voges–Proskauer positive, lysine decarboxylase positive, ornithine decarboxylase negative, and arginine dihydrolase negative belongs in the genus *Klebsiella*. However, inclusion of *K. mobilis* and *K. ornithinolytica* makes this classical identification more difficult at the genus level. In the following discussion, the genus *Enterobacter* will be considered without *E. aerogenes*.

Carbon source utilization tests are essential. All *Klebsiella* strains (with the exception of *K. pneumoniae* subsp. *ozaenae* and subsp. *rhinoscleromatis*) utilize the following six substrates: D-arabitol, *myo*-inositol, palatinose, quinate, D-sorbitol, and sucrose. No *Enterobacter*, *Pantoea*, or *Erwinia* species utilizes all six of these substrates. In the genus *Serratia*, only *S. ficaria* would utilize all six substrates and also L-arabitol and *i*-erythritol, which no *Klebsiella* has the simultaneous ability to utilize. Furthermore, D-adonitol is utilized by all *Klebsiella* strains except environmental strains of *K. pneumoniae*. In the genus *Enterobacter*, D-adonitol is utilized only by some strains of *Enterobacter cloacae*. Some strains of *Pantoea* also utilize D-adonitol.

Taxonomic Comments

The first organism of the genus *Klebsiella* described was a capsulated bacillus isolated from patients with rhinoscleroma (von Frisch, 1882). Then Friedländer (1882) described a bacterium from lungs of a patient who had died of pneumoniae. The organism was subsequently named *"Hyalococcus pneumoniae"* (Schroeter, 1889). The genus *Klebsiella* was coined by Trevisan (1885) to honor the German microbiologist Edwin Klebs (1834–1913). In 1887 (Trevisan, 1887), the genus contained *Klebsiella pneumoniae* (Friedländer bacillus) and *K. rhinoscleromatis* (von Frisch's bacillus). *"Bacillus mucosus ozaenae"*, which Abel (1893) observed in nasal secretion of patients with ozena, was later included in the genus as *K. ozaenae* (Bergey et al., 1925).

The genus *Klebsiella* with three species lasted up to the eighth edition of the *Bergey's Manual of Determinative Bacteriology*.

Flügge (1886) described *"Bacillus oxytocus perniciosus"* from old milk. This organism was named *"Aerobacter oxytocum"* (Bergey et al., 1923) and *Klebsiella oxytoca* (Lautrop, 1956). For many years, the existence of this indole-positive species was questioned and it was often considered a biogroup of *K. pneumoniae* (Edwards and Ewing, 1972; Ørskov, 1974). DNA relatedness studies showed *K. oxytoca* to be clearly distinct from *K. pneumoniae* (Jain et al., 1974; Brenner et al., 1977).

K. planticola (Bagley et al., 1981) was proposed for strains isolated primarily from botanical and soil environments (Bagley et al., 1981). *K. planticola* is distinct from other *Klebsiella* species based on DNA relatedness (Woodward et al., 1979; Izard et al., 1981a).

K. terrigena (Izard et al., 1981a), was proposed for strains isolated mainly from aquatic and soil environments (Izard et al., 1981a). It is a distinct species according to DNA relatedness studies (Woodward et al., 1979; Izard et al., 1981a).

The species described as *K. trevisanii* (Ferragut et al., 1983) was shown to be synonymous with *K. planticola* by DNA–DNA hybridization (Gavini et al., 1986).

In the first edition of the *Bergey's Manual of Systematic Bacteriology*, four species were recognized: *K. pneumoniae*, *K. oxytoca*, *K. planticola*, and *K. terrigena*.

K. ozaenae and *K. rhinoscleromatis* cannot be separated from *K. pneumoniae* by DNA relatedness (Brenner et al., 1972c). For this reason, *K. ozaenae* and *K. rhinoscleromatis* were treated as subspecies of *K. pneumoniae* in the first edition of the *Bergey's Manual of Systematic Bacteriology* (Ørskov, 1974).

For many years, *K. pneumoniae* could not be objectively separated from an organism observed by Escherich (1885), named *"Bacterium lactis aerogenes"* (Kruse, 1896), transferred to genus *"Aerobacter"* as *"A. aerogenes"*, and finally as *Enterobacter aerogenes* (Hormaeche and Edwards, 1960b). Confusion occurred since both *K. pneumoniae* and *"Aerobacter aerogenes"* fermented many carbohydrates (often with gas production), gave a positive Voges–Proskauer reaction, and reacted with *Klebsiella* capsular antisera. When Møller (1955) introduced the decarboxylase tests, *K. pneumoniae* was defined as nonmotile and ornithine decarboxylase negative, whereas *"A. aerogenes"* was defined as motile or nonmotile and ornithine decarboxylase positive (Hormaeche and Edwards, 1958). Later, a new genus *Enterobacter* was formed (Hormaeche and Edwards, 1960b) to which *"A. aerogenes"* was transferred as *E. aerogenes*.

Nomenclatural confusion occurred when a biogroup of *K. pneumoniae* was named *"Klebsiella aerogenes"* (Taylor et al., 1956). Cowan et al. (1960) subdivided *K. pneumoniae sensu lato* into *K. pneumoniae* (*sensu stricto*), *"K. aerogenes"*, *"K. edwardsii* subsp. *edwardsii*", and " *K. edwardsii* subsp. *atlantae*". Authentic or typical strains of *K. pneumoniae*, *K. edwardsii*, and *"K. aerogenes"* cannot be differentiated by DNA relatedness or protein electrophoresis, and thus belong to a single species, *K. pneumoniae* (Brenner et al., 1972c; Jain et al., 1974; Ferragut et al., 1989).

These problems around the epithet *aerogenes* were due to a lack of authentic cultures. Neotypes have since been designated when no original type strain was available.

Enterobacter aerogenes is much closer to *Klebsiella* species than to *Enterobacter cloacae* (the type species of that genus) based on phenotypic traits and DNA relatedness (Bascomb et al., 1971; Brenner et al., 1972c; Steigerwalt et al., 1976; Izard et al., 1980). The transfer of *E. aerogenes* to the genus *Klebsiella* has been proposed (Bascomb et al., 1971). However, since the name *"Klebsiella aerogenes"* had been used for another organism, a new name, *Klebsiella mobilis*, was coined (Bascomb et al., 1971).

Nonmotile, encapsulated, ornithine decarboxylase positive strains were described under the name *Klebsiella ornithinolytica*

(Sakazaki et al., 1989a). Apart from ornithine decarboxylase test, these strains are biochemically undistinguishable from *K. planticola*, and are identical to *K. planticola* by DNA hybridization (Farmer et al., 1985a; D.J. Brenner, personal communication). Farmer et al. (1985a) questioned the validity of *K. ornithinolytica* as a species distinct from *K. planticola*. Some ornithine decarboxylase positive strains have been shown to belong in *K. pneumoniae* by DNA relatedness criteria (Lindh and Frederiksen, 1990).

Finally, *Calymmatobacterium granulomatis*, which has not been cultured on bacteriological media, has been transferred to the genus *Klebsiella* as *Klebsiella granulomatis*, mostly based on *rrs* sequences (Carter et al., 1999). Partial *rpoB* sequencing suggested that *K. granulomatis* is very close to *K. pneumoniae* (Drancourt et al., 2001) and *phoE* sequencing showed *K. granulomatis* close to *K. pneumoniae* subsp. *rhinoscleromatis* (Carter et al., 1999). Thus, there is presently no proof that *K. granulomatis* is a species distinct from *K. pneumoniae*, and instead there are arguments to make it part of *K. pneumoniae*.

Sequence data are increasingly used to suggest or support nomenclatural changes and some of these changes can be anticipated. The taxonomic significance of the three sequence clusters observed in *K. pneumoniae* or the two sequence clusters in *K. oxytoca* (Brisse and Verhoef, 2001) is still unknown, and DNA–DNA hybridization data are needed before a proposal can be made to design new species or subspecies. Since the phylogenetic tree obtained by *rrs* gene (encoding 16S rRNA) sequence comparison showed the genus *Klebsiella* to be split in at least two groups, a new genus, *Raoultella*, was proposed to contain *R. planticola* (type species), *R. ornithinolytica*, and *R. terrigena* (Drancourt et al., 2001). More sequencing work needs to be done to evaluate this proposal since partial *rpoB* sequence failed to group *Raoultella* species in a single cluster and these sequences were only 512 nucleotides long.

Differentiation of the species of the genus *Klebsiella*

Table BXII.γ.229 presents the characteristics differentiating the species and subspecies of *Klebsiella*.

List of species of the genus Klebsiella

1. **Klebsiella pneumoniae** (Schroeter 1886) Trevisan 1887, 94[AL] (*Hyalococcus pneumoniae* Schroeter 1886, 1952.)
pneu.mo′ni.ae. Gr. n. *pneumonia* pneumonia, inflammation of the lungs; M.L. gen. n. *pneumoniae* of pneumonia.

   The characteristics are as described for the genus and as listed in Table BXII.γ.229. *K. pneumoniae* can be divided into three sequence clusters (Brisse and Verhoef, 2001) unrelated to the present subdivision in subspecies. *K. pneumoniae* is normally found in the intestinal tract of humans and animals. It may be isolated in association with several pathological processes in humans, e.g., community-acquired pneumonia or nosocomial urinary tract infection. Serotype K2 is the most common K-type isolated from patients with urinary tract infection, pneumonia, or bacteremia. In animals, *K. pneumoniae* may be isolated from metritis in mares, bovine mastitis, or generalized infections in captive monkeys. Environmental strains generally utilize more carbon sources than clinical strains, but often fail to utilize D-adonitol. Intraspecies DNA relative reassociation values among strains is ~80–90% (Brenner et al., 1972c) or 73–100% (Woodward et al., 1979).

   The mol% G + C of the DNA is: 56–58 (T_m) (Seidler et al., 1975).

   Type strain: ATCC 13883, CIP 82.9, DSM 30104, JCM 1662.

   GenBank accession number (16S rRNA): X87276, Y17656, AB004753, AF130981.

   a. **Klebsiella pneumoniae** *subsp.* **pneumoniae** (Schroeter 1886) Trevisan 1887, 94[AL] (*Hyalococcus pneumoniae* Schroeter 1886, 1952.)

      Distinguished from the subspecies subsp. *ozaenae* and subsp. *rhinoscleromatis* by the characteristics listed in Table BXII.γ.229. Clinical strains may produce an extended-spectrum β-lactamase.

      The mol% G + C of the DNA is: 56–58 (T_m) (Seidler et al., 1975).

      Type strain: ATCC 13883, CIP 82.9, DSM 30104, JCM 1662.

      GenBank accession number (16S rRNA): X87276, Y17656, AB004753, AF130981.

   b. **Klebsiella pneumoniae** *subsp.* **ozaenae** (Abel 1893) Ørskov 1984c, 355[VP] (Effective publication: Ørskov 1984b, 463) (*Klebsiella ozaenae* (Abel 1893) Bergey, Harrison, Breed, Hammer and Huntoon 1925, 266; *Bacillus mucosus ozaenae* Abel 1893, 167; *Bacillus ozaenae* (Abel 1893) Lehmann and Neumann 1896, 204.)
      o.zae′nae. L. fem. n. *ozaena* ozena; L. gen. n. *ozaenae* of ozena.

      Distinguished from the subspecies subsp. *pneumoniae* and subsp. *ozaenae* by the characteristics listed in Table BXII.γ.229. Occurs in ozena and other chronic diseases of the respiratory tract.

      The mol% G + C of the DNA is: not available.

      Type strain: ATCC 11296, CIP52.211, JCM 1663, LMG 3113.

      GenBank accession number (16S rRNA): Y17654, AF130982.

   c. **Klebsiella pneumoniae** *subsp.* **rhinoscleromatis** (Trevisan 1887) Ørskov 1984c, 355[VP] (Effective publication: Ørskov 1984b, 464) (*Klebsiella rhinoscleromatis* Trevisan 1887, 95; *Bacterium rhinoscleromatis* (Trevisan 1887) Migula 1900, 352.)

rhi.no.scle.ro′ ma.tis. M.L. adj. *rhinoscleromatis* pertaining to rhinoscleroma.

Distinguished from the subspecies subsp. *pneumoniae* and subsp. *ozaenae* by the characteristics listed in Table BXII.γ.229. Found in patients with rhinoscleroma.

The mol% G + C of the DNA is: not available.

Type strain: ATCC 13884, CIP 52.210, JCM 1664, LMG 3184.

GenBank accession number (16S rRNA): Y17657, AF130983.

2. **Klebsiella granulomatis** (Aragão and Vianna 1913) Carter, Bowden, Bastian, Myers, Sriprakash and Kemp 1999, 1698^{VP} (*Calymmatobacterium granulomatis* Aragão and Vianna 1913, 221.)
gran.u.lo′ ma.tis. L. dim. n. *granulum* a small grain; Gr. suff. *-oma* a swelling or tumor; M.L. n. *granuloma* a granuloma; M.L. gen. n. *granulomatis* of a granuloma.

Occurs in granuloma inguinale (donovanosis) lesions. The bacilli (Donovan bodies) are seen in large vacuolated macrophages found in dermal infiltrate of genital ulcerations. Grow in the yolk sac of developing embryo or in cell cultures (fresh mononuclear cells or Hep-2 cell line). No growth in bacteriological media. Type strain: No type culture is currently available.

The mol% G + C of the DNA is: not available.

GenBank accession number (16S rRNA): AF009171, AF010251, AF010252, AF010253.

3. **Klebsiella mobilis** Bascomb, Lapage, Willcox and Curtis 1971, 279^{AL}*
mo′ bi.lis. L. adj. *mobilis* movable, motile.

The characteristics are described in Table BXII.γ.229. This is the only motile species in the genus. Strains producing extended-spectrum β-lactamase occur as nosocomial pathogens. Occur in water, sewage, soil, dairy products, and the feces of humans and animals. Also an opportunistic pathogen.

The mol% G + C of the DNA is: 53–54 (Bd).

Type strain: ATCC 13048, CIP 60.86, DSM 30053, JCM 1235, LMG 2094.

GenBank accession number (16S rRNA): AB004748.

4. **Klebsiella oxytoca** (Flügge 1886) Lautrop 1956, 375^{AL} (*Bacillus oxytocus perniciosus* Flügge 1886, 268.)
oxy.to′ ca. Gr. *oxys* sour, acid; Gr. suff. *-tokos* bearer, producer; M.L. n. *oxytocus* acid-producer, spurious; M.L. adj. *oxytoca* (sic) acid-producing.

The characteristics are as described for the genus and as listed in Table BXII.γ.229. Present in the intestinal tract of humans and animals. Can be isolated from various pathological processes and from botanical and aquatic environments. *K. oxytoca* can be divided into two sequence clusters (Brisse and Verhoef, 2001). Encapsulated; typable with *Klebsiella* K antisera. The intraspecies DNA relative reassociation values were 75% in the study by Brenner et al. (1972c) and 95% (average value) in the study by Woodward et al. (1979).

The mol% G + C of the DNA is: 55–58 (T_m).

Type strain: ATCC 13182, CIP 103434, JCM 1665, LMG 3055.

GenBank accession number (16S rRNA): Y17655, AB004754, AF129440.

5. **Klebsiella planticola** Bagley, Seidler and Brenner 1982, 266^{VP} (Effective publication: Bagley, Seidler and Brenner 1981, 109.)*
plan.ti′ co.la. L. fem. n. *planta* a plant; L. suff. *-cola* dweller; M.L. fem. n. *planticola* plant-dweller.

The characteristics are as described for the genus and as listed in Table BXII.γ.229. Isolated mainly from botanical, aquatic, and soil environments. Three biovars have been described (Naemura et al., 1979). Encapsulated; typable with *Klebsiella* K antisera. The average intraspecies DNA relative reassociation value is above 75% (Woodward et al., 1979).

The mol% G + C of the DNA is: 53.9–55.4 (T_m) (Seidler et al., 1975).

Type strain: ATCC 33531, ATCC 33558, CIP 100751, DSM 3069, IFO 14939.

GenBank accession number (16S rRNA): X93215, Y17659, AB004755, AF129443.

6. **Klebsiella terrigena** Izard, Ferragut, Gavini, Kersters, De Ley and Leclerc 1981a, 125^{VP}
ter.ri.ge′ na. L. n. *terra* soil; L. suff. *gena* origin; M.L. n. *terrigena* from soil.

The characteristics are as described for the genus and as listed in Table BXII.γ.229. Isolated mainly from aquatic and soil environments. The average intraspecies DNA relative reassociation value is above 86% (Izard et al., 1981a).

The mol% G + C of the DNA is: 56.7 (T_m) (Izard et al., 1981a).

Type strain: ATCC 33257, CIP 80-07, DSM 2687, JCM 1687, LMG 3222.

GenBank accession number (16S rRNA): Y17658, AF129442.

Species Incertae Sedis

1. **Klebsiella ornithinolytica** Sakazaki, Tamura, Kosako and Yoshizaki 1989b, 495^{VP} (Effective publication: Sakazaki, Tamura, Kosako and Yoshizaki 1989a, 205.)
or.ni.thi.no.ly′ ti.ca. M.L. *ornithinum* ornithine, an amino acid; Gr. adj. *lyticus* dissolving; M.L. fem. adj. *ornithinolytica* ornithine dissolving.

The CDC group called Klebsiella Group 47 corresponds to this species (Sakazaki et al., 1989a). Physiological and nutritional characteristics are presented in Table BXII.γ.229. Isolated from clinical materials, including urine, sputa, stool, and pus. Can be found in food. Clinical significance unknown.

The mol% G + C of the DNA is: 57–58 (T_m) (Sakazaki et al., 1989a).

Type strain: ATCC 31898, CIP 103576, DSM 7464, JCM 6096.

GenBank accession number (16S rRNA): AF129441, AJ251467.

**Editorial Note: Klebsiella mobilis* Bascomb et al. 1971 and *Enterobacter aerogenes* Hormaeche and Edwards 1960b have the same type strain and therefore are homotypic synonyms.

**Editorial Note: Klebsiella trevisanii* Ferragut et al. 1983 (phenon K of Gavini et al., 1977) is a junior subjective synonym of *K. planticola* (Gavini et al., 1986).

Genus XVII. **Kluyvera** *Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981b, 382^{VP} (Effective publication: Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981a, 927)*

J.J. FARMER III

Kluy' ve.ra. M.L. fem. n. *Kluyvera* named by Asai et al. (1956) to honor the Dutch microbiologist A.J. Kluyver, who made many contributions to microbial physiology and taxonomy.

Small rod-shaped cells, 0.5–0.7 × 2–3 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile, with peritrichous flagella. Contains the enterobacterial common antigen. Facultatively anaerobic. Catalase positive (weak). Oxidase negative. Nonpigmented. Ferment, rather than oxidize, D-glucose and other carbohydrates. Reduce nitrate to nitrite. **Positive for indole production, methyl red, citrate utilization (Simmons), ornithine decarboxylase, motility at 36°C, growth in the presence of cyanide (KCN test), malonate utilization, and esculin hydrolysis. Ferment D-glucose, lactose, sucrose, D-mannitol, salicin, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, α-methyl-D-glucoside, melibiose, mucate, D-mannose, and D-galactose. Produce visible gas during fermentation. Produce large amounts of α-ketoglutaric acid during the fermentation of D-glucose.** Negative for Voges–Proskauer, H_2S production (TSI), urea hydrolysis, phenylalanine deaminase, arginine dihydrolase, gelatin hydrolysis (22°C), lipase (corn oil), DNase, and the fermentation of dulcitol, adonitol, *myo*-inositol, erythritol, and D-arabitol. Susceptible to colistin, sulfadiazine, gentamicin, kanamycin, tetracycline, and chloramphenicol (disk diffusion method on Mueller–Hinton agar); **resistant to carbenicillin; penicillin, ampicillin, and cephalothin**; variable susceptibility to nalidixic acid and streptomycin.

Some strains produce kluyveramycin, unusual reddish blue crystals of unknown composition. **Occur in human clinical specimens**, food, soil and sewage. Probably an occasional **opportunistic pathogen and a cause of extraintestinal infections of humans**.

The genus *Kluyvera* includes *Kluyvera ascorbata*, *K. cryocrescens*, and *K. georgiana*. There is also a fourth species with standing in nomenclature, *Kluyvera cochleae*, but it appears to be a synonym of *Enterobacter intermedius* (originally named *E. intermedium*; corrected by von Graevenitz, 1990).

The mol% G + C of the DNA is: 55.1–56.6.

Type species: **Kluyvera ascorbata** Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981b, 382 (Effective publication: Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981a, 927.)

FURTHER DESCRIPTIVE INFORMATION

Literature Since *Kluyvera* was described in 1956, there have been many reports in the literature; over 40 have been cataloged in MEDLINE and many others in BIOSIS. In addition to the genus and species names, "Enteric Group 8", *"K. citrophila"*, and *"K. noncitrophila"* should also be included as a search terms, although they are rarely used today.

The National Library of Medicine's Internet site Entrez (http://www.ncbi.nlm.nih.gov), which covers most of the sequence databases, includes full sequences of the 16S rRNA gene for *K. ascorbata*, *K. georgiana*, and *K. cochleae* (*Enterobacter intermedius*), and a partial sequences for *K. cryocrescens*. A search for the term *Kluyvera* yielded 29 sequences.

Problems in evaluating the literature Computer literature searches often yield articles such as "First case of bacteremia due to xx" or "Wound infections due to yy"; where "xx" and "yy" are newly described bacterial species. The reader should evaluate these reports very critically, with particular attention to the way that cultures were identified. Over half the cultures of new or unusual *Enterobacteriaceae* in these reports have been misidentified (Farmer unpublished). *Kluyvera* strains from two published studies were subsequently identified as *Serratia fonticola* (Enteric Reference Laboratory at CDC, unpublished).

Sources, strains, collections The original collection described by Farmer et al. (1981a) included isolates from body sites that are normally sterile such as blood (2 isolates) and urine (13). Strains were also from nonsterile body sites: intestine or feces (8), throat or sputum (40), and other or unspecified (10). Other isolates were from nonhuman sources or culture collections: cow (1), water (1), sewage (4), food (1), milk (1), soil (1), hospital sink (1), and unspecified or unknown (18). These other sources suggest ways that humans and animals might come in contact with the organism. The description of the genus was based on an unweighted tabulation of 193 *Kluyvera* strains from the Enteric Reference Laboratory's collection; 149 of *K. ascorbata*, 38 of *K. cryocrescens*, and six of *Kluyvera* species 3.

Occurrence in human feces The original report of Farmer et al. (1981a) listed eight isolates from human feces. All these presumably came from cases of diarrhea or intestinal infections, and were identified as *Kluyvera ascorbata*. Although clinical information that accompanied the cultures was limited, there was no suggestion of an etiological role for *Kluyvera*. Fainstein et al. (1982a) isolated strains of *Kluyvera* from patients with and without diarrhea, and in the title of their paper suggested that *Kluyvera* strains might have had a role in some of the diarrhea cases. Kay et al. (1990) isolated 28 *Kluyvera* strains during a 2-yr prospective case-control study of acute gastroenteritis in children younger that 2 years old, and concluded that *Kluyvera* did not appear to be a diarrheal pathogen. In summary, there is no strong evidence that *Kluyvera* strains can actually cause diarrhea or intestinal infections, but this issue warrants further study. The presence of *Kluyvera* in food and water is a possible source of these intestinal isolates.

Occurrence in extraintestinal human specimens: clinical significance Most of the *Kluyvera* strains have been from the respiratory tract. Forty strains from the original collection (Farmer et al., 1981a) were from throat or sputum. Clinical significance and the ability to actually cause infections are still being evaluated. Systematic study and additional case reports are needed. Schwach (1979) reported three isolates of Enteric Group 8 from upper respiratory tract specimens. The strains were in mixed culture and not detected in subsequent specimens; thus they were of doubtful clinical significance. Braunstein et al. (1980) reported two cases that yielded cultures identified as Enteric Group 8 (subsequently, both were identified as *K. ascorbata*). One of these was from the sputum of a 6-year-old boy with pulmonary tuberculosis, and was not considered clinically significant. A second isolate was from gallbladder drainage fluid of a 63-year-old

woman with acute pancreatitis. Based on chart review, this isolate was considered clinically significant. Of CDC's current collection of 144 *Kluyvera* strains, none has been isolated from spinal fluid, but five strains have been isolated from blood: three strains (two from France) of *K. ascorbata*, one strain of *K. cryocrescens* (a 3-month-old, at autopsy), and one of *Kluyvera* species group 3. Most of these were submitted with little or no patient information, so it was impossible to evaluate clinical significance. The five blood isolates and the growing number of literature reports (Aldová et al., 1985b; Wong, 1987; Luttrell et al., 1988; Thaller et al., 1988; Tristram and Forbes, 1988; Dollberg et al., 1990; Sierra-Madero et al., 1990; Yogev and Koszlowski, 1990; Sezer et al., 1996; Padilla et al., 1997) suggest that *Kluyvera* is more than a benign saprophyte. Most new species of *Enterobacteriaceae* have at least attained the status of "infrequent opportunistic pathogen". Based on present knowledge, this status also seems appropriate for *Kluyvera*. The respiratory tract has been the most common source for *Kluyvera*, but there is no strong evidence that it is clinically significant at this site (however, one isolate of *K. ascorbata* was from a lung at autopsy). The respiratory tract (particularly sputum) is notoriously difficult to evaluate for clinical significance. The urinary tract has been the next most common source, but it has also been difficult to document clinical significance (Tristram and Forbes, 1988).

Occurrence in other animals Bangert et al. (1988) identified strains as *Kluyvera* species along with other *Enterobacteriaceae* in a fecal culture survey of 47 captive raptors (the bird orders *Falconiformes* and *Strigiformes*).

Reporting of *Kluyvera* species Originally, Farmer et al. (1981a) were pessimistic that most clinical and public health laboratories could identify *Kluyvera* strains to species. They suggested that reference laboratories could attempt complete identification, but that clinical laboratories should not attempt the additional phenotypic testing with the addition of special tests. Normal identification methods should allow an identification to the genus level, with a report of "*Kluyvera* species". The addition of one new *Kluyvera* species, and seven new *Buttiauxella* species has made it even more difficult to identify cultures of *Kluyvera–Buttiauxella* to species. Further work is needed to find better tests for differentiation. In the meantime, a conservative approach would be to use terms such as "*Kluyvera* species", "*Buttiauxella* species", and "*Kluyvera–Buttiauxella* group" for cultures that cannot be definitively assigned a species name.

Accumulation of α-ketoglutaric acid Asai et al. (1956) assigned considerable importance to the fact that *Kluyvera* strains accumulated extremely large amounts of α-ketoglutaric acid during D-glucose fermentation. However, few other *Enterobacteriaceae* have been investigated for this property.

Enrichment and Isolation Procedures

Strain of *Kluyvera* are not difficult to grow and are typical *Enterobacteriaceae* in most respects. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility.

Differentiation of the genus *Kluyvera* from other genera

Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* gives the results for *K. ascorbata*, *K. cryocrescens*, and *K. georgiana* in 47 biochemical tests normally used for identification (Farmer, 1999). There are no genus- or species-specific tests or sequences for the identification of *Kluyvera*. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the section on the family *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 of that section. Several computer programs greatly facilitate analyzing the results.

Taxonomic Comments

Kluyvera is a genus with a turbulent history. Although the name was used in the literature from 1956 to 1980, the genus name, and the two original species names (*"K. citrophila"* and *"K. noncitrophila"*) did not appear on the 1980 Approved Lists of Bacterial Names. The reasons for these omissions were never stated. Thus, *Kluyvera*, *"K. citrophila"*, and *"K. noncitrophila"* lost standing in nomenclature. To resolve this nomenclatural problem, Farmer et al. (1981a) proposed a redefined genus *Kluyvera*.

The history of *Kluyvera* Asai et al., 1956 In 1956 and 1957, Asai and co-workers in Japan proposed the genus name *Kluyvera* for a group of "polarly flagellated" bacteria that produced large amounts of α-ketoglutaric acid during the fermentation of D-glucose. Five strains were originally studied, one from soil and four from sewage (Table BXII.γ.230). Two species names were proposed, *"K. citrophila"* and *"K. noncitrophila"*, which were based on the difference among the strains in utilizing citrate as the sole source of carbon and energy (Asai et al., 1956). Asai and co-workers state (based on their interpretation of Kluyver and van Niel, 1936) that the genus was named to honor Professor A.J. Kluyver, who, with C.B. van Niel, in 1936 postulated that there may be a group of polarly flagellated organisms in the tribe *Pseudomonadeae* that have a mixed acid type of fermentation similar to *Escherichia* (called *Bacterium* in the paper). If such a group were to be discovered, it could be a separate genus, which would differentiate it from the genus *Aeromonas*, which has a butylene glycol fermentative pathway rather than a mixed acid pathway. Asai and co-workers thought they had discovered this postulated group of polarly flagellated organisms and named the group *Kluyvera* in honor of A.J. Kluyver for his many contributions to microbial metabolism and physiology. *Kluyvera* was classified in the tribe *Pseudomonadeae*, which at that time included nonfermentative genera, but also included the fermenters of the genus *Aeromonas*. Today, the family *Pseudomonadaceae* is restricted to bacteria that do not ferment glucose.

***Kluyvera* Asai et al., 1956 was "abolished" in 1962** In 1962, Asai and co-workers confirmed the observations of J.M. Shewan and Rudolph Hugh that all five of their *Kluyvera* strains actually had peritrichous rather than polar flagella (Asai et al., 1962). Thus, they proposed an alternative classification; the two species *"K. citrophila"* and *"K. noncitrophila"* were "transferred" to the genus *Escherichia* in the family *Enterobacteriaceae*. Thus, this alternative classification would result in the creation of two "new combinations"—*"Escherichia citrophila"* and *"Escherichia noncitrophila"*; however, these names were rarely used and did not appear on the 1980 Approved Lists of Bacterial Names.

History of the redefined genus *Kluyvera*: *Kluyvera* Farmer et al., 1981a The Enteric Reference Laboratory's interest in this group of organisms began in 1977 when the laboratory's

TABLE BXII.γ.230. Current nomenclature and classification (Farmer et al., 1981a) for strains originally proposed as *"Kluyvera citrophila"* and *"K. noncitrophila"*

Original name given by Asai et al. (1962)	Current (proposed) classification	ATCC number	Original strain designation	Source
"K. citrophila"	*K. ascorbata*	14236	6,β	Sewage, Japan
"K. citrophila"	*K. cryocrescens*	14237	84C,α	Soil, Tokyo, Japan
"K. citrophila"	*K. cryocrescens*	14238	11,γ	Sewage, Keihin District, Japan
"K. noncitrophila"	*K. cryocrescens*	14239	4	Sewage, Keihin District, Japan
"K. noncitrophila"	*K. cryocrescens*	14240	10	Sewage, Keihin District, Japan

STRAIN MATCHER computer programs listed over a dozen strains with almost identical biochemical reactions that had been reported as "unidentified". During this period, groups of unidentified *Enterobacteriaceae* were being defined, and this was the eighth group. Thus, the vernacular name "Enteric Group 8" was given to the group, and strains were then reported with this designation and included a comment that additional strains would be welcomed so the group could be further studied. Soon after Enteric Group 8 was defined, Holmes of the Computer Identification Laboratory, National Collection of Type Cultures (NCTC), England, reported that the NCTC collection had strains that were almost identical to our Enteric Group 8, and they had been cataloged under the name *Kluyvera* (unpublished). The NCTC stains of *Kluyvera* were then studied at CDC and the conclusions of Holmes were confirmed. Since then, Enteric Group 8 was thought of as a synonym of *Kluyvera*. The DNA–DNA hybridization studies of Fanning et al. (1979) defined two large species groups in *Kluyvera* and defined a third group of strains that became "*Kluyvera* species group 3", and was thought to represent one or more additional *Kluyvera* species. Although *Kluyvera* was "abolished" in 1962 (Asai et al., 1962), reports in the literature continued to use the name *Kluyvera*, probably because strains had been deposited in the American Type Culture Collection, NCTC, and other culture collections. The name *Kluyvera* was proposed as a redefined genus by Farmer et al. (1981a) because this was considered a better alternative than proposing a new genus. The main consideration was that the name *Kluyvera* was still being used in the literature, and that the genus *Kluyvera* was well represented in culture collections by established strains of long standing.

Phylogenetic position Based on both phenotype and DNA–DNA hybridization, Farmer et al. (1981a) thought that *Kluyvera* was a tight, well-defined genus of *Enterobacteriaceae*. Two 16S rDNA trees (Figure BXII.γ.198 in the section on *Budvicia* and Figure BXII.γ.189 in the chapter on the family *Enterobacteriaceae*) include *Kluyvera*. Unfortunately, the species of *Kluyvera* and *Buttiauxella* were not included in the 16S rDNA tree published by Spröer et al. (1999).

Additional species of *Kluyvera* There are probably several additional *Kluyvera* species. The Enteric Reference Laboratory's collection has 21 strains that were reported as "*Kluyvera* species", three as "possible-probable" *Kluyvera ascorbata*, three as *Kluyvera cryocrescens*, and two as "*Kluyvera–Buttiauxella* Group" (Enteric Reference Laboratory, 2000, unpublished). These 29 and the following strains need to be studied by techniques that measure evolutionary relatedness: the four additional strains included in *Kluyvera georgiana* by Müller et al. (1996); the three strains of *K. ascorbata* and five strains of *K. cryocrescens* that had divergence values in DNA–DNA hybridization experiments in the range 5–9 (see Table 4 of Farmer et al., 1981a); and all other "atypical strains" of *Kluyvera* and *Buttiauxella*. Detailed study of this collection would probably result in the definition of many new species.

Further studies needed New phenotypic and molecular tests are needed for the routine identification and differentiation of *Kluyvera*, *Buttiauxella*, and related organisms. The 16S rDNA sequences of all *Kluyvera* and *Buttiauxella* species need to be completed or repeated and new trees drawn. This could provide a useful way to identify strains and clarify relationships of the genera and species.

Further Reading

Asai, T., H. Iizuka and K. Komagata. 1964. The flagellation and taxonomy of the genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains. J. Gen. Appl. Microbiol. *10*: 95–126.

Asai, T., S. Okumura and T. Tsunoda . 1956. On a new genus, *Kluyvera*. Proc. Japan Acad. *32*: 488-493.

Fainstein, V., R.L. Hopfer, K. Mills and G.P. Bodey. 1982. Colonization by or diarrhea due to *Kluyvera* species. J. Infect. Dis. *145*: 127.

Farmer, J.J. III, G.R. Fanning, G.P. Huntley-Carter, B. Holmes, F.W. Hickman, C. Richard and D.J. Brenner. 1981. *Kluyvera*: a new (redefined) genus in the family *Enterobacteriaceae*. Identification of *Kluyvera ascorbata* sp. nov. and *Kluyvera cryocrescens* sp. nov. in clinical specimens. J. Clin. Microbiol. *13*: 919–933.

Luttrell, R.E., G.A. Rannick, J.L. Soto-Hernandez and A. Verghese. 1988. *Kluyvera* species soft tissue infection: case report and review. J. Clin. Microbiol. *26*: 2650–3651.

Differentiation of the species of the genus *Kluyvera*

The original two named species of *Kluyvera*, *K. ascorbata* and *K. cryocrescens*, had almost identical results in the 47 tests commonly used to identify cultures of *Enterobacteriaceae* (Farmer et al., 1981a; see Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*). However, these species can be differentiated by several simple phenotypic tests (Table BXII.γ.231). *K. georgiana* is also almost identical to *K. ascorbata* and *K. cryocrescens* in the 47 tests commonly used to identify cultures of *Enterobacteriaceae* (Farmer et al., 1981a), which is one of the reasons it was not originally named. At present there are no simple ways to differentiate it.

TABLE BXII.γ.231. Tests useful for differentiating the three *Kluyvera* species; and between *Kluyvera* and *Buttiauxella*[a]

Test or property	*K. ascorbata*	*K. cryocrescens*	*K. georgiana*	*Buttiauxella*
Ascorbate test	97	0	nd	nd
Growth and D-glucose fermentation at 5°C within 21 d	3	100	nd	nd
Zone of inhibition around carbenicillin and cephalothin (see Figure 4 of Farmer et al., 1981a)	Small	Large	nd	nd
Growth on cefsulodin-irgasan-novobiocin agar[b]	100	0	nd	nd
Minimum inhibitory concentration (MIC) for irgasan (μg/ml)[b]	>128	≤0.25	nd	nd
Lysine decarboxylase (Møller)	97	23	100	−
Gas production during the fermentation of D-glucose	93	95	17	+
Dulcitol fermentation	25	0	33	0
Indole production	92	90	100	−
Sucrose fermentation	98	81	100	0
Citrate utilization	96	80	100	d
Raffinose fermentation	98	100	100	d
α-Methyl-D-glucoside fermentation	98	95	100	−
Melibiose fermentation	99	100	100	d
Acetate utilization	50	86	83	0

[a]Symbols: +, most strains are positive; −, most strains are negative; d, species to species variation, but many strains are negative. Each number is the percentage positive after 2 d incubation at 36°C.

[b]The data for growth on CIN agar and the irgasan MIC are from Altwegg et al. (1986); the other data are from Farmer et al. (1981a) and Farmer (1999).

List of species of the genus Kluyvera

1. **Kluyvera ascorbata** Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981b, 382[VP] (Effective publication: Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981a, 927.)
a.scor.ba′ta. ascorbate, a modern chemical term, a salt of ascorbic acid; M.L. fem. adj. *ascorbata* pertaining to ascorbate.

   The characteristics are as described for the genus. See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for the results of 47 biochemical tests normally used for identification (Farmer, 1999). Occurs in human clinical specimens, water, sewage, and food. Probably an opportunistic pathogen of humans that is occasionally encountered in clinical microbiology laboratories. The type strain was isolated from human sputum.

   The mol% G + C of the DNA is: 56.1–56.6 (Bd).

   Type strain: ATCC 33433, CDC 0648-74.(holotype).

   GenBank accession number (16S rRNA): AF176560.

   Additional Remarks: The ATCC includes two other strains; ATCC 33434 and ATCC 14236 (Table BXII.γ.230).

2. **Kluyvera cryocrescens** Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981b, 382[VP] (Effective publication: Farmer, Fanning, Huntley-Carter, Holmes, Hickman, Richard and Brenner 1981a, 927.)
cry.o.cres′cens. Gr. n. *kryos* cold; L. fem. pres. part. *crescens* growing; M.L. fem. adj. *cryocrescens* growing in the cold; referring to the fact the cultures grow at 4–5°C.

   The characteristics are as described for the genus. See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for the results of 47 biochemical tests normally used for identification (Farmer, 1999). Occurs in human clinical specimens, soil, water, sewage, and the hospital environment. Probably an opportunistic pathogen of humans that is rarely encountered in clinical microbiology laboratories.

   The mol% G + C of the DNA is: 55.1 (Bd).

   Type strain: ATCC 33435, CDC 2065-78 (holotype).

   Additional Remarks: The ATCC includes six other strains, four of which are described in Table BXII.γ.230.

3. **Kluyvera georgiana** Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 63[VP]
geor.gi.a′na. M.L. fem. adj. *georgiana* pertaining to Georgia, U.S.A., where the characterization and redefinition of *Kluyvera* was done.

   Synonym: *K. georgiana* and *Kluyvera* species group 3 of Farmer et al. (1981a) are similar in several ways, but are different in circumscription as described below.

   The characteristics are as described for the genus. See Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* for the results of 47 biochemical tests normally used for identification (Farmer, 1999). Currently, it is not possible to completely differentiate *K. georgiana* from *K. ascorbata* and *K. cryocrescens* with simple tests (Table BXII.γ.231). The type strain was isolated from human sputum.

   Phenotypic properties as originally given by Müller et al. (1996). The species includes the type strain but no other strains. Other strains named *K. georgiana* include CDC 2774-70 (ATCC 51703), CDC 2065-76 (ATCC 51702), CDC 4246-74, and CDC 3108-76 (Müller et al., 1996). However, no data are presented to show that these four strains are highly related to the type strain by either DNA–DNA hybridization

or phenotype (see the discussion below of *Kluyvera* species 3). Thus, there is a change in circumscription between the two species. In the future, additional strains that are highly related to the type strain should be included.

The mol% G + C of the DNA is: not determined.

Type strain: 189, ATCC 51603, CDC 2891-76, DSM 9409 (holotype).

Other Organisms

1. *Kluyvera cochleae* Müller, Brenner, Fanning, Grimont and Kämpfer 1996, 63[VP]

coch' le.ae. L. fem. gen. n. *cochleae* of a snail.

Müller et al. (1996) defined a fourth *Kluyvera* species, *Kluyvera cochleae*, which now appears to be a junior subjective synonym of *Enterobacter intermedius*. *K. cochleae* included two strains isolated from snails in Germany and one strain from a slug in Great Britain. In DNA–DNA hybridization experiments (Müller et al., 1996), the type strain of *K. cochleae* S3/1-49[T] was labeled and was highly related (86–92% at 60°C with divergence values of 0.5–1.0) to the two other strains. It was 19–24% related to strains of *Buttiauxella*, but no relatedness values were given for its relationship to the type strains of the three *Kluyvera* species or to other *Enterobacteriaceae*.

Farmer (unpublished, see Farmer, 2003) studied the three strains of *K. cochleae* and found that they apparently metabolize D-glucose via the butanediol pathway (i.e., they are Voges–Proskauer positive). This is a very unlikely pathway to occur in *Kluyvera*, which was defined to be methyl red positive (well documented to accumulate large amounts of α-ketoglutaric acid) and Voges–Proskauer negative. Computer analysis of the phenotypic data indicated that the strains of *K. cochleae* were essentially identical to 10 reference strains of *Enterobacter intermedius* (Farmer, unpublished). *Enterobacter* is a genus that is well known to have the butanediol pathway of D-glucose metabolism (Voges–Proskauer positive). Later it was shown that the type strains of these two species have almost identical published 16S rRNA sequences. These observations led to further laboratory studies (Brenner and Steigerwalt, unpublished; Farmer, unpublished). By DNA–DNA hybridization, the type strains of the two species are very highly related (D.J. Brenner, personal communication).

To summarize, all current data indicate that *Kluyvera cochleae* and *Enterobacter intermedius* are different names for the same organism. The two species have different type strains, so they should be considered as subjective synonyms, with *Enterobacter intermedius* being the senior subjective synonym, since it was validly published first.

2. *"Kluyvera fluvialis"*

In an abstract, Gadaleta et al. (1996) from Buenos Aires, Argentina, described their work with "strain 21g" isolated from a polluted river. They determined its 16S rDNA sequence, compared it to the sequences of *K. ascorbata* and *K. cryocrescens*, and concluded " . . . the 21g strain is a member of an until now undescribed species of *Kluyvera* that we propose to be named *K. fluvialis*". Since the name *"Kluyvera fluvialis"* has not been effectively published or validly published, it lacks standing in nomenclature.

The vernacular name *Kluyvera* species group 3 was originally given by Farmer et al. (1981a) to five strains (2774-70, 2065-76, 2891-76, 4246-74, and 3108-76) that were 60–68% related (with divergence values of 9–12%) by DNA–DNA hybridization to strains of *K. ascorbata* and *K. cryocrescens* (see Table 4 in Farmer et al., 1981a). None of these strains were labeled and tested against the others to determine if they all belonged to the same species. However, the authors concluded that the five strains represented one or more additional species of *Kluyvera*. Because of its uncertain circumscription and the lack of simple tests to differentiate it from the two named *Kluyvera* species, they did not give it a scientific name, designate a type strain, or deposit strains in the American Type Culture Collection.

One of the strains (2891-76) was later designated as the type strain of *Kluyvera georgiana* by Müller et al. (1996) as described above. *K. georgiana* of Müller et al. (1996) also include the other four strains that Farmer et al. (1981a) included in *Kluyvera* species 3. However, no information is included in the paper of Müller et al. (1996) to show that these latter four strains are highly related to the type strain of *K. georgiana* by DNA–DNA hybridization or phenotype. The assignment of these four strains to *K. georgiana* or a new *Kluyvera* species awaits further study.

Genus XVIII. **Leclercia** *Tamura, Sakazaki, Kosako, and Yoshizaki 1987, 179[VP] (Effective publication: Tamura, Sakazaki, Kosako, and Yoshizaki 1986, 183)*

CAROLINE M. O'HARA AND J.J. FARMER III

Le.clerc' i.a. M.L. fem. n. *Leclercia* named to honor H. Leclerc, a French bacteriologist, who first described and named this organism *Escherichia adecarboxylata* in 1962, and who made many other contributions to enteric bacteriology.

Small rod-shaped cells, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile at 36°C and 25°C with peritrichous flagella. Facultatively anaerobic. Catalase positive. Oxidase negative. **Many strains produce a nondiffusible yellow pigment that may be weak and may be lost on storage and subculture.** Ferment, rather than oxidize, D-glucose and other carbohydrates. Reduce nitrate to nitrite. **Positive for indole production, methyl red, growth in the presence of cyanide, malonate utilization, esculin hydrolysis, ONPG, and the fermentation of lactose, D-mannitol, dulcitol, salicin, adonitol, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, erythritol, esculin, melibiose, D-arabitol, mucate, D-mannose, and D-galactose. Produce visible gas during fermentation.** Negative for Voges–Proskauer, citrate utilization (Simmons), H_2S production (TSI), phenylalanine deaminase, **lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase**, gelatin hydrolysis (22°C), lipase (corn oil) production, DNase production, and the fermentation of *myo*-inositol, D-sorbitol, α-methyl-D-glucoside, and erythritol.

Most strains are susceptible to colistin, nalidixic acid, sulfadiazine, gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, carbenicillin, cephaloridine, and ampicillin (disk diffusion method on Mueller–Hinton agar); **resistant to penicillin**.

Isolated from human clinical specimens that are normally sterile such as blood and urine. Also isolated from other clinical specimens such as sputum and wounds, usually in mixed culture. Probably an **opportunistic pathogen that occasionally causes extraintestinal infections** in humans. Occasionally occurs in human feces, but there is **no evidence that it causes diarrhea or intestinal infections**. Natural reservoirs and ecological niches are not completely defined, but has been isolated from water, food, milk, and other environmental samples. Appears to be important in the processing of "dry-cured" hams. A rarely isolated genus of *Enterobacteriaceae*.

The mol% G + C of the DNA is: 52–55.

Type species: **Leclercia adecarboxylata** (Leclerc 1962) Tamura, Sakazaki, Kosako, and Yoshizaki 1987, 179 (Effective publication: Tamura, Sakazaki, Kosako, and Yoshizaki 1986, 183) (*Escherichia adecarboxylata* Leclerc 1962, 737.)

Further descriptive information

Literature Since the genus was described in 1986, there have been a few reports in the literature; seven cataloged in MEDLINE and 18 cataloged in BIOSIS. These reports include reviews of new *Enterobacteriaceae*; human clinical specimens and infections; occurrence in animals, plants, food, water, and the environment; taxonomic studies; and studies on basic biology such as physiology-metabolism, biochemistry, and genetics.

***Leclercia* strains in reference laboratory collections** The original report by Tamura et al. (1986) described 58 isolates from human clinical specimens including blood, urine, sputum, and feces, and 27 isolates from nonhuman sources, including food, water, and the environment. However, they did not specify the number of isolates from each of these sources. Farmer et al. (1985a) described their studies with the type strain (isolated from water), and concluded that their collection also contained 6–10 isolates that were very similar. The CDC collection now includes 18 human isolates: blood (5), urine (1), wound (1), sputum (1), hand wound (1), leg wound (2), finger (1), ear (1), feces (3), and unspecified (2). It also includes 11 isolates from nonhuman sources or culture collections: intravenous fluid bottle (1), milk (1), environmental (1), and unspecified or unknown (8).

Occurrence in human clinical specimens Strains of *L. adecarboxylata* have been from a wide variety of human clinical specimens, but there have been few systematic clinical studies or case reports. Temesgen et al. (1997) found only five strains during 1984–1995 at the Mayo Clinic in Rochester, Minnesota, USA. In three cases it was isolated from wounds of the lower extremities, all in mixed culture. Another case was a pneumonia patient whose sputum yielded *L. adecarboxylata* along with other organisms. One patient had a culture of *Leclercia adecarboxylata* that was clinically significant. A 35-year-old woman with acute nonlymphocytic leukemia had undergone bone marrow transplantation. She developed bacteremia and fever while she was neutropenic, and *L. adecarboxylata* was isolated in pure culture from one of two sets of blood cultures.

Other cases of bacteremia have also been described. Daza et al. (1993) reported the case of a 45-year-old alcoholic who had severe abdominal pain, hypotension, tachycardia, and diaphoresis 6 hours after undergoing paracentesis for the removal of ascitic fluid. Four days after undergoing a laparotomy, he developed fever and had three blood cultures positive for *L. adecarboxylata*. Otani and Bruckner (1991) reported a case in an 8½-month-old with a history of congenital gastroschisis and intestinal atresia. He came to the emergency department with shaking chills and fever about 1 hour after total parenteral nutrition through a central line catheter. The blood culture from the central line was positive for *L. adecarboxylata*. Dudkiewicz and Szewczyk (1993) described one isolate of *L. adecarboxylata* in their series of 72 cases of bacterial endocarditis.

When *L. adecarboxylata* is isolated in mixed culture from specimens such as sputum and wounds, it is difficult to determine clinical significance. Martinez et al. (1998) reported its isolation from an ulcer exudate. However, the isolation of this organism from blood cultures suggests that it is at least an opportunistic pathogen. Additional studies and case reports are needed to better define its role as a human pathogen.

Occurrence in human feces Several collections have included isolates from feces (Tamura et al., 1986), which usually are from patients with diarrhea. Cai et al. (1992) described three patients in China with diarrhea whose fecal cultures were positive for *L. adecarboxylata*, but the authors could not demonstrate an etiological role. Although it has occasionally been isolated from feces, there is no evidence that *L. adecarboxylata* can actually cause diarrhea or infections of the intestinal tract.

Occurrence in animals, plants, food, water, and the environment In addition to sources previously mentioned, *L. adecarboxylata* (referred to as Enteric Group 41 by Bangert et al., 1988) also has been isolated from captive raptors (the bird order *Falconiformes*; the falcons) (Bangert et al., 1988). *L. adecarboxylata* appears to be important in the processing of "dry-cured" hams. It was the only species present at the end of the fast-curing process, and predominated along with some other *Enterobacteriaceae* at the end of the slow-curing process (Marin et al., 1996). These environmental reservoirs suggest additional ways that hospital and other patients can come in contact with *L. adecarboxylata*. If a blood culture from a patient on total parenteral nutrition is positive for *L. adecarboxylata*, all the liquids should be cultured as possible reservoirs.

Isolation, identification, culture preservation, phenotypic characterization Strains of *Leclercia adecarboxylata* grow well on media normally used in enteric bacteriology, and are typical *Enterobacteriaceae* in most respects. The typical yellow pigment is probably present in most cultures on initial isolation, but is easily lost. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, and antibiotic susceptibility. Table BXII.γ.232 below summarizes the phenotypic characteristics of *L. adecarboxylata*. Also see Table BXII.γ.193 of the chapter on the family *Enterobacteriaceae* that has the tabulated biochemical reactions of *L. adecarboxylata* and other *Enterobacteriaceae*.

Taxonomic Comments

History Leclerc (1962) recognized a group that he referred to as "yellow-pigmented coliform bacteria" that was similar to *E. coli* in its IMViC reactions, but differed by producing a yellow pigment and fermenting many sugars. He proposed that it be recognized as a separate species in the genus *Escherichia*, *Escher-*

TABLE BXII.γ.232. Phenotypic characteristics of *Leclercia adecarboxylata* based on data from three different laboratories

Test or property	Study 1[a]	Study 2	Study 3
Number of strains studied	20	85	33
Incubation temperature[b]	30°C	35°C	36°C
Final reading for the test[b]	2 d	2 d	2 d
Yellow pigment production, 2 d	60[c] (30°C)	nd	37° (25°C)
Yellow pigment production, 7 d	nd	14 (25°C)	63 (25°C)
Indole production	90	100	100
Methyl red	nd	100	100
Voges–Proskauer	0	0	0
Citrate utilization (Simmons)	0	0	0
H_2S production	0	0 (KIA)	0 (TSI)
Urea hydrolysis	70	10	48
Phenylalanine deaminase	0	0	0
Lysine decarboxylase	0	0	0
Arginine dihydrolase	0	0	0
Ornithine decarboxylase	0	0	0
Motility, 35–37°C	nd	99	79
Motility, 22–25°C	nd	nd	60
Motility, 30°C	100	nd	nd
Gelatin hydrolysis	0	0	0 (22°C)
KCN test (% resistant)	100	100	97
Malonate utilization	100	100	93
D-Glucose, acid production	100	100	100
D-Glucose, gas production	100	99	97
Lactose fermentation	85	100	93
Sucrose fermentation	20	52	66
D-Mannitol fermentation	100	100	100
Dulcitol fermentation	70	87	86
Salicin fermentation	100	100	100
Adonitol fermentation	55	98	93
myo-Inositol fermentation	nd	0	0
D-Sorbitol fermentation	8	9	0
D-Arabinose fermentation	100	100	100
Raffinose fermentation	30	59	66
L-Rhamnose fermentation	100	100	100
Maltose fermentation	100	100	100
D-Xylose fermentation	100	100	100
Trehalose fermentation	100	100	100
Cellobiose fermentation	100	100	100
α-Methyl-D-glucoside fermentation	0	0	0
Erythritol fermentation	0	0	0
Esculin hydrolysis	100	100	100
Melibiose fermentation	100	100	100
D-Arabitol fermentation	nd	96	96
Glycerol fermentation	100 (30 d)		3
Mucate fermentation	90	65	93
Tartrate fermentation (Jordan)	70	nd	83
Acetate utilization	nd	0	28
Lipase (corn oil)	nd		0
DNase production	0	0 (25°C)	0 (25°C)
Nitrate reduction to nitrite	100	99	100
Oxidase	nd	0	0
ONPG test	100	100	100
D-Mannose fermentation	100	100	100
Tyrosine hydrolysis	nd	nd	0
D-Galactose fermentation	100	100	100
MacConkey agar, growth	nd	nd	100
Catalase	nd	nd	+
Citrate, Christensen	nd	0	0
H_2S on peptone iron agar	nd	nd	0
Kauffmann–Peterson tests:			
Citrate	nd	0	nd
D-Tartrate	0	0	nd
L-Tartrate	30	nd	nd
meso-Tartrate	0	nd	nd
Growth at 4–5°C	15 (30 d)	49 (14 d)	nd
Growth at 35–37°C	nd	nd	+
Growth at 41°C	85	nd	nd
Growth at 42°C	nd	0	nd
Growth at 50°C	0	nd	nd
β-Glucuronidase	0	0	nd
Pectinase	nd	0	nd
Tetrathionate reductase	25	54	nd
β-Xylosidase	95	100	nd
Carbon source utilization:			
Number of compounds tested	123	122	nd
Number (%) utilized	26 (21.1)	31 (25.4)	nd
Amygdalin fermentation	nd	0	nd
D-Arabinose fermentation	nd	0	nd
L-Arabitol fermentation	nd	0	nd
Arbutin fermentation	nd	100	nd
D-Fructose fermentation	100	nd	nd
D-Fucose fermentation	nd	0	nd
L-Fucose fermentation	nd	0	nd
Gentiobiose fermentation	nd	100	nd
D-Gluconate fermentation	nd	100	nd
2-Ketogluconate fermentation	nd	100	nd
5-Ketogluconate fermentation	nd	0	nd
Glycogen fermentation	5	nd	nd
Inulin fermentation	nd	0	nd
D-Levulose fermentation	nd	100	nd
D-Lyxose fermentation	nd	0	nd
L-Lyxose fermentation	nd	0	nd
Melizitose fermentation	0	0	nd
Methylmannoside fermentation	nd	0	nd
D-Ribose fermentation	100	100	nd
L-Sorbose fermentation	0	0	nd
D-Tagatose fermentation	nd	0	nd
D-Turanose fermentation	nd	0	nd
Xylitol fermentation	nd	0	nd
Tween 80 hydrolysis	nd	0	nd

[a]Study 1, Izard et al. (1985); study 2, Tamura et al. (1986); study 3, Farmer (2003).

[b]The standard conditions used in each study; a different temperature or time for the final reading is indicated in parentheses. Abbreviations: TSI, triple sugar iron agar; KIA, Kligler iron agar; ONPG, o-nitrophenyl-β-D-galactopyranoside.

[c]Each number is the percentage of strains that are positive. Most positive reactions occur during the first 24 h. For a few tests in study there were not enough quantitative data, so these results are represented by symbols with the usual meaning stated in this *Manual*; nd, not determined.

ichia adecarboxylata. Ewing and Fife (1972) studied the type strain of *Escherichia adecarboxylata* and proposed a different classification. They classified it in their redefined species (which is now known to be heterogeneous) *Enterobacter agglomerans* as "a typical strain of biogroup G3".

Escherichia adecarboxylata* as a distinct group of *Enterobacteriaceae*; proposal of *Leclercia Several other investigators considered the taxonomic position of *E. adecarboxylata* and concluded that it is different from *Escherichia*, the *Enterobacter agglomerans–Erwinia* complex, and from other *Enterobacteriaceae* (see Table BXII.γ.233) (Gavini et al., 1983b; Sakazaki et al., 1983; Izard et al., 1985; Verdonck et al., 1987). The situation was clarified when Tamura et al. (1986) used DNA–DNA hybridization to study their collection of strains. They showed that *E. adecarboxylata* was different from other taxa of *Enterobacteriaceae*. The type strain of *E. adecarboxylata* was more than 70% related to eight other strains and was 64–66% related to two additional strains. It was only 32% related to the type strain of *Enterobacter agglomerans* and only 26% related to the type strain of *Escherichia coli*. Because it was distinct by both DNA–DNA hybridization and phenotype, they proposed a new genus *Leclercia* with one species *Leclercia adecarboxylata* to

include strains formerly classified as *Escherichia adecarboxylata*. Their proposal is an alternative classification that has been well accepted by the scientific community.

Phylogeny based on 16S rDNA sequencing Recently a complete 16S rDNA sequence of "strain LBV 449" (which is not the type strain) was deposited (GenBank accession number AJ276393) (De Baere et al., 2001). A 16S rRNA tree that includes *L. adecarboxylata* can be found in the chapter on the family *Enterobacteriaceae*. The 16S rDNA sequencing data agree with data from DNA–DNA hybridization experiments that *Leclercia* is distinct from other genera of *Enterobacteriaceae* (Tang et al., 1998). Unfortunately, *L. adecarboxylata* was not included in the tree published by Spröer et al. (1999).

Several sequences related to mercury resistance have also been deposited.

TABLE BXII.γ.233. Differentiation of *Leclercia adecarboxylata* from its closest phenotypic relatives in *Enterobacteriaceae*

Test	*Leclercia adecarboxylata*	*Escherichia coli*	"*Enterobacter agglomerans* complex"[a]
Indole production	+	+	d
Methyl red	+	+	d
Lysine decarboxylase	−	+	−
Arginine dihydrolase	−	d	−
Ornithine decarboxylase	−	d	−
Yellow pigment	d	−	d
KCN	+	−	d
D-Sorbitol fermentation	−	+	d
Cellobiose fermentation	+	−	d
D-Glucose, gas	+	+	d
Dulcitol fermentation	+	d	d
D-Arabitol fermentation	+	−	−

[a]See Farmer (2003) for a more detailed description of this "vernacular name". It is a term defined for practical identification in the clinical microbiology laboratory. It includes over a dozen DNA—DNA hybridization groups that were originally included in the species *Enterobacter agglomerans*, which is now known to be a heterogeneous species.

Further Reading

Leclerc, H. 1962. Étude biochemique d' *Enterobacteriaceae* pigmentées. Ann. Inst. Pasteur (Paris) *102*: 726–741.

Izard, D., J. Mergaert, F. Gavini, A. Beji, K. Kersters, J. De Ley and H. Leclerc. 1985. Separation of *Escherichia adecarboxylata* from the *Erwinia herbicola-Enterobacter agglomerans* complex and from the other enterobacteriaceae by nucleic acid and protein electrophoretic techniques. Ann. Inst. Pasteur Microbiol. *136B*: 151–168.

Tamura, K., R. Sakazaki, Y. Kosako and E. Yoshizaki. 1986. *Leclercia adecarboxylata*, gen. nov., comb. nov., formerly known as *Escherichia adecarboxylata*. Curr. Microbiol. *13*: 179–184.

Temesgen, Z., D.R. Toal and F.R. Cockerill, III. 1997. *Leclercia adecarboxylata* infections: case report and review. Clin. Infect. Dis. *25*: 79–81.

List of species of the genus Leclercia

1. **Leclercia adecarboxylata** (Leclerc 1962) Tamura, Sakazaki, Kosako, and Yoshizaki 1987, 179[VP] (Effective publication: Tamura, Sakazaki, Kosako, and Yoshizaki 1986, 183) (*Escherichia adecarboxylata* Leclerc 1962, 737; *Enterobacter agglomerans* biogroup G3 Ewing and Fife 1972, 10.)
a.de.car.box.y.la′ta. Gr. adj. *a* without; M. Fr. n. *decarboxyl* removal of a molecule of carbon dioxide from an organic compound; *adecarboxylata* without decarboxylase activity; because it has negative reactions in lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase; i.e., "triple decarboxylase negative".

   The characteristics are as described for the genus; a more complete description is given in Table BXII.γ.232. Isolated from human clinical specimens, environmental samples, food, and water. Its clinical significance is not fully documented but its potential role as a pathogen is suggested by isolates from blood and similar specimens that are normally sterile. However, it may be colonizing rather than infecting nonsterile body sites. The isolates from food, drinking water, feces, and an intravenous fluid bottle suggest ways that humans come in contact with it. There is no evidence that it can cause diarrhea or intestinal infections. It should be considered a rarely isolated species of *Enterobacteriaceae*, and a possible opportunistic pathogen (extraintestinal infections only) for humans. The type strain was isolated from drinking water by Leclerc, 1962.

   The mol% G + C of the DNA is: 52–55 (T_m).

   Type strain: 1783, ATCC 23216, CIP 82.92, DSM 5077, HAMBI 1696, JCM 1667, LMG 2803.

   Additional Remarks: The American Type Culture Collection includes 7 other strains of *L. adecarboxylata*, including four strains from "apple fruit".

Other Organisms

Currently *Leclercia* has only one species, but it will be interesting to see if future studies will change or clarify the current concept or circumscription of the genus. Tamura et al. (1986) included only one species in *Leclercia*; however, Table 2 of their paper includes strains 1523 and 363, which were only 66.2% and 64.2% related to the type strain by DNA–DNA hybridization; 70% is the usual cutoff point for the level of species. They did not include divergence values (ΔT_m), so it is more difficult to determine if these two strains are as related to the type strain as the other eight strains. The CDC collection contains five strains that were identified as "possible-probable" *Leclercia adecarboxylata* because their phenotypic characteristics were similar to, but different from, strains now included in the species. These five strains, the two of Tamura et al. (1986), and similar strains should be investigated as possible additional species of *Leclercia*.

Genus XIX. **Leminorella** *Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985c, 375^{VP} (Effective publication: Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985b, 235)*

J.J. Farmer III and Frances W. Brenner

Le.mi.no.rel' la. M.L. dim. *-ella* ending; M.L. fem. n. *Leminorella* named to honor Leon Le Minor, a French microbiologist, for his many contributions to enteric bacteriology including the nomenclature, classification, and serotyping of *Salmonella*; lysogeny; metabolic plasmids; and new and rapid biochemical tests. The name also honors Simone Le Minor, who also made many contributions to enteric bacteriology as head of the National *Salmonella* Centre of France and for her research on *Serratia* serotyping.

Small rod-shaped cells, conforming to the general definition of the family *Enterobacteriaceae*. Gram-negative. **Nonmotile at 36°C and 25°C.** Contain the enterobacterial common antigen. Facultatively anaerobic. Catalase positive (strong and rapid). Oxidase negative. Nonpigmented. Ferment, rather than oxidize, D-glucose. Reduce nitrate to nitrite. **Inactive biochemically. Positive for H_2S production (TSI and PIA), tyrosine hydrolysis, and fermentation of L-arabinose, D-xylose, and L-tartrate.** Negative for indole production, Voges–Proskauer, urea hydrolysis, phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, growth in the presence of cyanide (KCN test), malonate utilization, esculin hydrolysis, gelatin hydrolysis (22°C), lipase (corn oil), DNase, and the fermentation of lactose, sucrose, D-mannitol, salicin, adonitol, *myo*-inositol, D-sorbitol, raffinose, L-rhamnose, maltose, trehalose, cellobiose, α-methyl-D-glucoside, erythritol, melibiose, D-arabitol, glycerol, D-mannose, and D-galactose. Susceptible to colistin, nalidixic acid, sulfadiazine, gentamicin, kanamycin, tetracycline, chloramphenicol; **resistant to streptomycin, penicillin, ampicillin, carbenicillin, and cephalothin** (disk diffusion method on Mueller-Hinton agar).

Usually isolated from feces, but there is no evidence that strains cause diarrhea or intestinal infections. Some strains agglutinate in *Salmonella* diagnostic antisera, and **can be misidentified as being *Salmonella*. Rarely isolated from other clinical specimens.** Ecological niche may be the human intestinal tract since there are only a few isolates from other clinical specimens, and no reported isolates from animals, food, water or the environment. **A very rarely isolated genus of *Enterobacteriaceae*.**

The mol% G + C of the DNA is: not reported.

Type species: **Leminorella grimontii** Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985c, 375 (Effective publication: Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985b, 235.)

Further descriptive information

Literature Since the genus was described in 1985, there have been only a handful of reports in the literature, six cataloged in MEDLINE and 11 cataloged in BIOSIS. Except for the original report (Hickman-Brenner et al., 1985b), none of these has dealt specifically with *Leminorella*. The literature reports that have mentioned *Leminorella* include reviews and taxonomic studies of new *Enterobacteriaceae* (Schindler, et al., 1992; Gilchrist, 1995; Aleksic and Bockemühl, 1999); evaluation of commercial identification products or "kits" for *Enterobacteriaceae* (Kitch et al., 1994); and studies on physiology, metabolism, or biochemistry that included *Leminorella* strains (Bouvet and Grimont, 1987a; Satta et al., 1988; Bouvet et al., 1989; Grimont and Bouvet, 1989; Hodinka et al., 1991; Thaller et al., 1995; Hamana, 1996). In addition to the genus and species names, "Enteric Group 57" should be included as a search term in computerized literature searches, although it is rarely used today.

Sources and clinical significance Nine of the original *Leminorella* isolates were from feces, and two were from urine. Since the original paper in 1985, the CDC Enteric Reference Laboratory has received and identified nine additional human isolates, seven from feces and one each from urine and a decubitus wound. The clinical significance of *Leminorella* strains in these extraintestinal specimens is unknown, and further study and case reports are needed. Although strains of *Leminorella* have occasionally been isolated from feces of people with diarrhea, there is no evidence that it can actually cause diarrhea or intestinal infections. Its interest for clinical microbiology and public health appears to be more as a nuisance because *Leminorella* strains might be misidentified as *Salmonella* unless more complete biochemical and serological testing is done.

Original misidentification as *Salmonella* Several of the isolates from feces had been referred to the CDC's *Salmonella* reference laboratory because the strains agglutinated one or more *Salmonella* antisera. Presumably these tests were done with commercial antisera and with "live", rather than alcohol-treated, cultures, and the referring laboratory could not completely serotype the strain or confirm an identification as *Salmonella*. Four *L. grimontii* strains and one *L. richardii* strain have been reported to cross-react when tested at CDC with alcohol-treated antigens and CDC reference antisera. One of the strains agglutinated very weakly in O group B serum and in single factor O27 serum. The other strains did not agglutinate.

Isolation, identification, culture preservation, phenotypic characterization Strains of *Leminorella* grow on media normally used in enteric bacteriology, but they grow more slowly and are slower or more inactive biochemically than typical *Enterobacteriaceae*. One strain of *L. grimontii* and all four strains of *L. richardii* were negative for D-glucose fermentation after incubation for 24 h, but all became positive at 48 h (Hickman-Brenner et al., 1985b). On MacConkey agar at 24 h, strains appear as small colonies that are colorless (lactose negative). See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility.

Biochemical reactions Table BXII.γ.234 summarizes the biochemical reactions of the two *Leminorella* species. The tests useful for differentiation of *L. grimontii* and *L. richardii* are given in Table BXII.γ.235. Compared to other species of *Enterobacteriaceae*, strains of *Leminorella* are weak and inactive biochemically. One important characteristic is hydrogen sulfide production. Within 48 h on triple sugar iron agar, the species give an alkaline slant and a weak acid reaction in the butt with H_2S production.

Antibiotic susceptibility In their original description, Hickman-Brenner et al. (1985b) gave the zone sizes for each of the

TABLE BXII.γ.234. Biochemical reactions of *Leminorella grimontii* and *L. richardii* based on strains studied by DNA–DNA hybridization

Characteristic	*Leminorella grimontii*	*Leminorella richardii*
Number of strains	6[a]	4[a]
Indole production	0[b]	0
Methyl red	100	0
Voges–Proskauer	0	0
Citrate utilization (Simmons)	100	0
H_2S production (TSI[c])	100	100
H_2S production (PIA)	100	100
Urea hydrolysis	0	0
Phenylalanine deaminase	0	0
Lysine decarboxylase	0	0
Arginine dihydrolase	0	0
Ornithine decarboxylase	0	0
Motility at 36°C	0	0
Gelatin hydrolysis (22°C)	0	0
Growth in KCN	0	0
Malonate utilization	0	0
D-Glucose, acid production at 24 h	83	0
D-Glucose, acid production at 48 h	100	100
D-Glucose, gas production	33 (100)	0
Lactose fermentation	0	0
Sucrose fermentation	0	0
D-Mannitol fermentation	0	0
Dulcitol fermentation	83	0
Salicin fermentation	0	0
Adonitol fermentation	0	0
myo-Inositol fermentation	0	0
D-Sorbitol fermentation	0	0
L-Arabinose fermentation	100	100
Raffinose fermentation	0	0
L-Rhamnose fermentation	0	0
Maltose fermentation	0	0
D-Xylose fermentation	83	100
Trehalose fermentation	0	0
Cellobiose fermentation	0	0
α-Methyl-D-glucoside fermentation	0	0
Erythritol fermentation	0	0
Esculin hydrolysis	0	0
Melibiose fermentation	0	0
D-Arabitol fermentation	0	0
Glycerol fermentation	17 (33)	0
Mucate fermentation	100	50 (75)
Tartrate fermentation (Jordan's)	100	100
Acetate utilization	0	0
Lipase (corn oil)	0	0
DNase production (25°C)	0	0
Nitrate reduction to nitrite	100	100
Oxidase	0	0
ONPG test	0	0
Yellow pigment (25°C)	0	0
D-Mannose fermentation	0	0
Tyrosine hydrolysis	83 (100)	75 (100)
D-Galactose fermentation	0	0

[a]These are the original strains that were studied by DNA–DNA hybridization and used by Hickman-Brenner et al. (1985b) to define the species.

[b]Each number gives the percentage positive after 2 d incubation at 36°C (unless a different time or temperature is indicated; phenylalanine, oxidase, and nitrate reduction are done only at 1 d). The cumulative % positive between 3 and 7 d is given for some tests in parentheses. Some of the percentages are slightly different from those given in the original descriptions (Hickman-Brenner et al., 1985b) and are based on tests results that were repeated after the original publication.

[c]TSI, triple sugar iron agar; PIA, peptone iron agar; ONPG, o-nitrophenyl-β-D-galactopynanoside.

TABLE BXII.γ.235. Differentiation of *Leminorella grimontii* and *L. richardii*[a]

Test	Incubation time, days	*L. grimontii*	*L. richardii*
Methyl red	2	100	0
Citrate utilization (Simmons)	2	100	0
D-Glucose, acid production	1	83	0
D-Glucose, gas production	7	100	0
Dulcitol fermentation	2	83	0
Glucosaminidase activity[b]		100	0

[a]These data are based on results in Table BXII.γ.234 which included only strains that were documented to belong to these two species by DNA–DNA hybridization (Hickman-Brenner et al., 1985b). Each number gives the percentage positive at the specified indicated period; all the biochemical tests were done at 36°C.

[b]Data from Hodinka et al. (1991).

11 strains with the 12 different antibiotics that have been used for many years in their reference laboratory as a aid in identification. There was little variation among the three *Leminorella* species, and all 11 strains were resistant to streptomycin, penicillin, ampicillin, carbenicillin, and cephalothin. Although this is not a unique pattern, the antibiogram can be a useful additional way of differentiating *Leminorella* from other genera of *Enterobacteriaceae*.

DNA, RNA, and protein sequences There is a full sequence (bases 1 to 1482) of the 16S rRNA gene of *L. grimontii* listed in GenBank (Spröer et al., 1999).

A 16S rDNA tree that includes *Leminorella grimontii* can be found in the chapter on the family *Enterobacteriaceae* (Fig. BXII.γ.189). The 16S rDNA sequencing data agree with data from DNA–DNA hybridization that *Leminorella* is distinct from other genera of *Enterobacteriaceae*. In the tree, *Pragia fontium* is the closest relative, in agreement with the tree published by Spröer et al. (1999) with respect to these two genera being on a distinct branch. However, the two trees differ in the placement of other organisms.

Differentiation of the genus *Leminorella* from other genera

Strains of *Leminorella* can be distinguished because they do not ferment D-mannose, but hydrolyze tyrosine, much like strains of *Proteus*; however, they are negative for urea hydrolysis and phenylalanine deaminase. They differ from *Salmonella* because they are lysine and ornithine decarboxylase negative and do not ferment D-sorbitol. Also see Table BXII.γ.193 of the chapter on the family *Enterobacteriaceae*), which gives the percentage positive for 47 biochemical tests done at the Enteric Reference Laboratories at CDC with standard media and methods. These tests have proved useful for identification and for differentiating *Leminorella* from other species in the family.

Taxonomic Comments

History and discovery In 1980 one of us (JJF, unpublished) noticed two H_2S^+ strains that seemed to be distinct from other *Enterobacteriaceae*, and coded these as a "possible new group of *Enterobacteriaceae*" in the laboratory's master computer database. In 1981 diagnostic culture 1944-81 was received and studied in more detail (Hickman-Brenner et al., 1985b). It was from a stool culture of a 7-month-old patient in Hawaii with gastroenteritis. The culture had a low identification score for other *Enterobacteriaceae* in the computer program GEORGE (Farmer et al., 1985a). However, the computer program STRAIN MATCHER listed nine additional strains that had been reported as "unidentified" that were very similar biochemically. Several of these strains had been sent to CDC because they reacted weakly in *Salmonella* antisera. Five strains had been sent as *Salmonella*, or with a diagnosis of suspected salmonellosis or diarrhea; two were sent as "possible

Citrobacter"; and one was a "suspect H_2S^+ *Shigella*" (Hickman-Brenner et al. 1985b). The vernacular name Enteric Group 57 was coined in 1981 (Hickman-Brenner and Farmer, unpublished; see Farmer et al., 1985a) for the original group of 10 strains, and the taxonomic position of the group was unknown. Enteric Group 57 was simply thought of as being a unique group of H_2S-producing strains that was being confused with *Salmonella* in some reference laboratories. An 11th strain was received in 1982.

Proposal of the genus *Leminorella* Hickman-Brenner et al. (1985b) used DNA–DNA hybridization (hydroxyapatite, $^{32}PO_4$) to further characterize Enteric Group 58. Strain 1944-81^T was labeled and was highly related (77–97% at 60°C with divergence values of 0–0.5%) to five other Enteric Group 58 strains. Strain 1944-81 was only 3–16% related to other species of *Enterobacteriaceae*. Because it was distinct from all *Enterobacteriaceae* by both DNA–DNA hybridization and phenotype, it was given a scientific name. The group of six strains was named *Leminorella grimontii*, which was designated as the type species for the genus *Leminorella*. Five other strains were 32–60% related to 1944-81, so strain 0978-82 was chosen from this group and tested by DNA hybridization against all 11 strains. Three strains were 93–94% related to strain 0978-82 with divergence values of 0.5–1.0. This group of four strains was named *Leminorella richardii*. The remaining strain 3346-72 was 60% and 40% related to strains 1944-81 and 0978-82, respectively. It was considered to be a third *Leminorella* species, that was closer to *L. grimontii*, and was given the vernacular name "*Leminorella* species 3". Since there was only one strain, it was not given a scientific name. Although *Leminorella* species 3 was distinct by DNA hybridization, it could not be distinguished from *L. grimontii* by simple tests (Hickman-Brenner et al., 1985b). Since the original report in 1985, additional strains of *Leminorella* have been studied biochemically, but not by DNA–DNA hybridization. *Leminorella* and its two named species gained standing in nomenclature in July 1985, when the names appeared on Validation List 18 (Hickman-Brenner et al., 1985c).

Problems in routine identification In the original publication DNA hybridization was used to divide the strains into two named species, and a description was written based on this clear separation (Tables BXII.γ.234 and BXII.γ.235). Unfortunately, *Leminorella* species 3 cannot be distinguished from *L. grimontii* with simple tests (Hickman-Brenner et al., 1985b). This strain illustrates the difficulty in identifying *Leminorella* cultures to the species level without the benefit of DNA–DNA hybridization. Since the original report in 1985, the Enteric Reference Laboratory has received 10 additional strains of *Leminorella*. Based on the differential reactions listed in Table BXII.γ.235, five of these were identified as *L. grimontii*; one as a "possible-probable" *L. grimontii* and four as *L. richardii*. Five of the strains identified as *L. grimontii* had at least one test that was in disagreement with the composite results in Table BXII.γ.234. The strains identified as *L. richardii* were more typical, and agreed with the definition given in Table BXII.γ.235, except that one strain had one atypical reaction. Thus, the identification of new strains of *Leminorella* to the genus level is probably correct, but the identification to the species level is more tentative and needs confirmation by DNA–DNA hybridization or other methods. Enzyme profiles (Hodinka et al., 1991) and whole-cell protein patterns (Schindler et al., 1992) appear promising for differentiation. Further study and better and simpler methods are needed to assist routine identification.

Further Reading

Hickman-Brenner, F.W., M.P. Vohra, G.P. Huntley-Carter, G.R. Fanning, V.A.I. Lowery, D.J. Brenner and J.J. Farmer III. 1985. *Leminorella*, a new genus of *Enterobacteriaceae*: identification of *Leminorella grimontii* sp. nov. and *Leminorella richardii* sp. nov. found in clinical specimens. J. Clin. Microbiol. *21*: 234–239.

List of species of the genus Leminorella

1. **Leminorella grimontii** Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985c, 375^{VP} (Effective publication: Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985b, 235) *gri.mon' ti.i.* M.L. gen. n. *grimontii* named to honor Patrick Grimont and Francine Grimont, French microbiologists at the Pasteur Institute for their many contributions to enteric bacteriology.

   The characteristics are as given for the genus. The biochemical reactions are given in more detail in Table BXII.γ.234, and tests for the differentiation of *L. grimontii* and *L. richardii* are given in Table BXII.γ.235. Isolated from human feces, but there is no evidence that it can cause diarrhea or intestinal infections. Rarely isolated from other clinical specimens. Its clinical significance in these extra-intestinal specimens needs further study. One isolate was from mouse feces. The type strain was isolated from the feces of a 7-month-old child with gastroenteritis.

   The mol% G + C of the DNA is: not reported.

   Type strain: ATCC 33999, CDC 1944-8, DSM 5078.

   GenBank accession number (16S rRNA): AJ233421.

   Additional Remarks: The American Type Culture Collection includes four other strains documented to be *L. grimontii* by DNA–DNA hybridization: ATCC 43006 (CDC 3257-77), from human feces, California; ATCC 43005 (CDC 3244-76), from human urine of an 80-year-old woman with a urinary tract infection, Hawaii; ATCC 43007 (CDC 3595-77), from urine of a 9-year-old girl, Pennsylvania; and ATCC 43008 (CDC 0301-79), from mouse feces, Maryland.

2. **Leminorella richardii** Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985c, 375^{VP} (Effective publication: Hickman-Brenner, Vohra, Huntley-Carter, Fanning, Lowery, Brenner and Farmer 1985b, 235.) *ri.char' di.i.* M.L. gen. n. *richardii* named to honor Claude Richard, a French microbiologist at the Pasteur Institute for his many contributions to enteric bacteriology.

   The characteristics are as given for the genus. The biochemical reactions are given in more detail in Table BXII.γ.234, and the differentiation of *L. richardii* from *L. grimontii* is given in Table BXII.γ.235. Isolated from human feces, but there is no evidence that it can cause diarrhea or intestinal infections. Nonhuman strains have not been reported. The type strain was isolated from the feces of a patient in Texas who had diarrhea. She also had systemic lupus erythematosus, and was on corticosteroid therapy. No other enteric pathogens were present.

The mol% G + C of the DNA is: not reported.

Type strain: ATCC 33998, CDC 0978-82.

Additional Remarks: The American Type Culture Collection includes three other strains documented to be *L. richardii* by DNA–DNA hybridization; all were from human feces in the USA: ATCC 43009 (CDC 598-78), Indiana; ATCC 43010 (CDC 2209-80), Pennsylvania; and ATCC 43011 (CDC 2502-80), Texas.

Other Organisms

1. "Leminorella species 3"

   In addition to the two named species of *Leminorella*, Hickman-Brenner et al. (1985b) also described a third species. Since there was only one strain, and because it could not be differentiated from *L. grimontii* by phenotypic tests, they gave it the vernacular name " *Leminorella* species 3".

   The reference strain, ATCC 43012, was isolated from the human feces, Georgia, USA. This is the only strain of species 3, and we suggest that it be designated the type strain if species 3 is given a scientific name.

   Deposited strain: ATCC 43012, CDC 3346-72.

Strains studied since 1985 have been reported without the benefit of DNA hybridization. One strain (1201-84) was reported as a "possible-probable" *Leminorella grimontii*. Five were reported as *L. grimontii* because they were more active biochemically. However, four of these had one or more atypical test results for this species. All new strains of *Leminorella* should be studied by DNA hybridization or other methods to determine their correct taxonomic position.

Genus XX. **Moellerella** *Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984c, 355[VP] (Effective publication: Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984a, 462)*

J.J. Farmer III and Frances W. Brenner

Moe.ller.el'la. M.L. dim. ending *-ella*; M.L. fem. n. *Moellerella* named to honor Vagn Møller for his contributions to enteric bacteriology, especially for Moeller media for the determination of lysine decarboxylase, ornithine decarboxylase, and arginine dihydrolase, that are widely used for the identification of *Enterobacteriaceae*.

Small rod-shaped cells, conforming to the general definition of the family *Enterobacteriaceae*. Contain the enterobacterial common antigen. Gram negative. Nonmotile. Facultatively anaerobic. Catalase positive. Oxidase negative. Nonpigmented. Ferment, rather than oxidize, D-glucose and other carbohydrates. Reduce nitrate to nitrite. **Positive for methyl red, citrate utilization (Simmons), ONPG, and the fermentation of lactose, sucrose, adonitol, raffinose, melibiose, D-arabitol, D-mannose, and D-galactose.** Negative for indole production, Voges–Proskauer, H_2S production (TSI), urea hydrolysis, phenylalanine deaminase, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, motility at 36°C, malonate utilization, esculin hydrolysis, gelatin hydrolysis (22°C), lipase (corn oil), and DNase, and the fermentation of dulcitol, salicin, *myo*-inositol, D-sorbitol, L-arabinose, L-rhamnose, D-xylose, trehalose, cellobiose, α-methyl-D-glucoside, erythritol, glycerol, and mucate. No visible gas produced during fermentation.

Most strains are susceptible to nalidixic acid, gentamicin, streptomycin, kanamycin, chloramphenicol, and cephalothin (disk diffusion method on Mueller-Hinton agar); **resistant to colistin, penicillin, ampicillin, and carbenicillin**. Variable susceptibility to sulfadiazine and tetracycline. Rarely isolated from human clinical specimens that are normally sterile. Most isolates have been from human feces, but there is **no evidence that it actually causes diarrhea or intestinal infections**. Natural reservoirs and ecological niches are not known. Can occur in water and food. **A rarely isolated genus of *Enterobacteriaceae* that is probably an opportunistic pathogen.**

The mol% G + C of the DNA is: not determined.

Type species: **Moellerella wisconsensis** Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984c, 355 (Effective publication: Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984a, 462.)

Further descriptive information

Literature Since *Moellerella* was described in 1984, there have been only a few reports in the literature, 11 cataloged in MEDLINE and 12 cataloged in BIOSIS. In addition to the genus and species names, "Enteric Group 46" should also be included as a search term in computerized literature searches, although it is rarely used today. The literature reports with *M. wisconsensis* include reviews and taxonomic studies of new *Enterobacteriaceae* (Farmer et al., 1985a; Richard, 1989; Gilchrist, 1995; Pokhil, 1996; Aleksic and Bockemühl, 1999); evaluation of commercial identification products or "kits" for *Enterobacteriaceae* (Gonzalez et al., 1986; Kitch et al., 1994); and surveys or comparisons of the family *Enterobacteriaceae* for metabolic pathways (Bouvet et al., 1989), acid phosphatases (Thaller et al., 1995), siderophores (Rabsch and Winkelmann, 1991), and polyamines (Hamana, 1996).

Occurrence in human feces Strains of *M. wisconsensis* were first recognized because of six strains submitted to CDC from Wisconsin; all were from human feces. Eight of the nine original cultures described by Hickman-Brenner et al. (1984b) were from feces and the other one was from water. Most isolates of *M. wisconsensis* have been from people with diarrhea, but there is no evidence that the strains are actually causing diarrhea or intestinal infections (Hickman-Brenner et al., 1984a). Marshall et al. (1986) reported the isolation of three strains of *M. wisconsensis* from 400 stool specimens screened for this organism with the aid of a new selective medium, developed in their laboratory. There was one report of *M. wisconsensis* isolation in the U.K., but its etiological role was not established in any of the diarrhea cases.

The interest of *M. wisconsensis* to clinical microbiology and public health laboratories may be more as a possible nuisance because strains have been picked as "suspect *Salmonella*" or "suspect *Yersinia*". More complete biochemical and serological testing should easily rule out these incorrect identifications.

Occurrence in extraintestinal human specimens Wallet et al. (1994) reported the isolation of *M. wisconsensis* from a bronchial aspirate of a patient with inhalation pneumonia. It was from an autopsy culture of a patient admitted in a deep coma following cardiac arrest. They concluded that the origin of the organism may have been from digestive secretions that inoculated the lower respiratory tract. This was the first isolate of *M. wisconsensis* from a human source other than stool or gallbladder. Wittke et al. (1985) reported the isolation of *M. wisconsensis* from the infected gallbladder of a 71-year-old man with typical signs of acute cholecystitis. Equal numbers of enterococci were also isolated. Serum from the patient taken on the 24th day after cholecystectomy did not contain agglutinating antibodies to heated or unheated suspensions of *M. wisconsensis*, providing negative evidence for its pathogenic role. Ohanessian et al. (1987) reported the isolation of *M. wisconsensis* along with *Hafnia alvei* from the infected gallbladder of a 77-year-old woman with coronary cardiac failure and gallstones. In 1985 the CDC's Enteric Reference Laboratory received the first isolate from human blood.

Clinical significance of human isolates The clinical significance and the ability of *M. wisconsensis* to actually cause infections should be carefully evaluated in cases that yield this new organism. Systematic study and good case reports are needed. The isolates described above suggest that it is probably an opportunistic pathogen with only a slight capacity to cause extraintestinal infections in humans.

Occurrence in other animals Bangert et al. (1988) isolated *M. wisconsensis* and other *Enterobacteriaceae* in a fecal culture survey of 47 captive raptors (the bird orders *Falconiformes* and *Strigiformes*). Giordano-Dias et al. (1997) reported isolates from the sandfly *Lutzomyia longipalpis* (*Diptera*: *Psychodidae*) maintained in the laboratory.

Occurrence in food and water One of the original nine strains reported by Hickman-Brenner et al. (1984a) was from a routine drinking water sample from a South Dakota (USA) town that did not chlorinate its water. Cabadajova and Kudrna (1988) of the Czech Republic isolated five strains of *M. wisconsensis* from food, but could not show an etiological role in relation to the corresponding human diarrhea cases.

Isolation, identification, culture preservation, phenotypic characterization Strains of *Moellerella* are not difficult to grow, and are typical *Enterobacteriaceae* in most respects. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility. Strains grow on media normally used in enteric bacteriology. On MacConkey agar, colonies of *M. wisconsensis* are bright red with precipitated bile around them, and thus are indistinguishable from *Escherichia coli* colonies (Hickman-Brenner et al., 1984a). Marshall et al. (1986) reported the enhanced isolation of *M. wisconsensis* from stool specimens with a selective medium developed in their laboratory that contained bacitracin and polymyxin.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* gives the results for *Moellerella* in 47 biochemical tests normally used for identification (Farmer, 1999). There are no genus- or species-specific tests or sequences for the identification of *Moellerella*. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the section on the family *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 in that chapter. Several computer programs greatly facilitate analyzing the results.

Antibiotic susceptibility In their original description, Hickman-Brenner et al. (1984a) gave the zone sizes for each of the nine strains around 12 different antibiotics (the standard "antibiogram" that has been used for many years in their reference laboratory as an aid in identification). There was some variation among the strains, but all were resistant to colistin and penicillin. Although this is not a unique pattern, the antibiogram can be a useful additional way of differentiating *M. wisconsensis* from other species of *Enterobacteriaceae*.

Taxonomic Comments

Discovery and DNA–DNA hybridization In 1980 the vernacular name Enteric Group 46 was applied (Hickman-Brenner et al., 1984b) to a group of strains that had been studied at the Centers for Disease Control, and most had originally been sent from the Wisconsin State Laboratory of Hygiene. The strains were characterized biochemically and were phenotypically distinct from all of the described organisms in the family *Enterobacteriaceae*. By DNA–DNA hybridization the strains were 78–97% related to the type strain, with divergence values (ΔT_m) of 0–1.5. Other *Enterobacteriaceae* were only 2–32% related. Because Enteric Group 46 was distinct by both DNA–DNA hybridization and phenotype, a new genus *Moellerella* with a single species, *Moellerella wisconsensis*, was proposed (Hickman-Brenner et al., 1984a).

Further Reading

Hickman-Brenner, F.W. , G.P. Huntley-Carter, Y. Saitoh, A.G. Steigerwalt, J.J. Farmer, III and D.J. Brenner. 1984. *Moellerella wisconsensis* a new genus and species of *Enterobacteriaceae* found in human stool specimens. J. Clin. Microbiol. *19*: 460–463.

Marshall, A.R., I.J. Al Jumaili and A.J. Bint. 1986. The isolation of *Moellerella wisconsensis* from stool samples in the U.K. J. Infect. *12*: 31–33.

Wittke, J.W., S. Aleksic and H.H. Wuthe. 1985. Isolation of *Moellerella wisconsensis* from an infected human gallbladder. Eur. J. Clin. Microbiol. *4*: 351–352.

List of species of the genus Moellerella

1. **Moellerella wisconsensis** Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984c, 355[VP] (Effective publication: Hickman-Brenner, Huntley-Carter, Saitoh, Steigerwalt, Farmer and Brenner 1984a, 462.)
wis.con.sen'sis. M.L. fem. adj. *wisconsensis* pertaining to the state of Wisconsin, U.S.A., where most of the original strains were isolated.

The characteristics are as given for the genus and are summarized in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Isolated from human feces but rarely from extraintestinal specimens, also isolated from animals, water, and food. The type strain was isolated from the stool culture of a 16-year-old girl in Wisconsin.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 35017, CDC 2896-78, DSM 5076.

Additional Remarks: The American Type Culture Collection includes four other strains, all from human feces.

Genus XXI. **Morganella** *Fulton 1943, 81*[AL]

J. Michael Janda and Sharon L. Abbott

Mor.ga.nel'la. M.L. dim. ending *-ella*; M.L. fem. n. *Morganella* named after H. de R. Morgan, who first studied the organism.

Straight rods, 0.6–0.7 × 1.0–1.7 μm. Gram negative. Motile by means of peritrichous flagella. Facultatively anaerobic. **Oxidative deamination of various amino acids including L-phenylalanine and L-tryptophan** (Singer and Volcani, 1955). **Urease positive. Indole positive** (Penner, 1984). Relatively few carbohydrates fermented. **Ornithine decarboxylase positive. Acid produced from D-mannose but not D-xylose. Gelatin not degraded.** The genus *Morganella* presently resides in the class *Gammaproteobacteria* in the family *Enterobacteriaceae*, based upon DNA hybridization data and not rDNA sequencing (Stackebrandt et al., 1988). 16S rDNA sequence data are presently unavailable.

The mol% G + C of the DNA is: 50 (Falkow et al., 1962).

Type species: **Morganella morganii** (Winslow, Kliger and Rothberg 1919) Fulton 1943, 81 (*Bacillus morgani* (sic) Winslow, Kliger and Rothberg 1919, 481; *Proteus morganii* (Winslow, Kliger and Rothberg 1919) Yale 1939b, 435.)

Further descriptive information

M. morganii displays morphologic characteristics typical of members of the *Enterobacteriaceae* appearing as short, straight rods. Capsules not produced (McKell and Jones, 1976). Peritrichous flagella with normal and curly curvature have been described (Leifson et al., 1955). Normal flagella have a wavelength of 2.09–2.36 μm with an amplitude of 0.51 μm. Curly flagella possess a shorter periodicity (1.14–1.18 μm) and amplitude (0.37–0.40 μm). Strains often express multiple fimbriae consisting of morphologically thin (outside diameter [o.d.] 4–5 nm) and thick forms (o.d. 7–8 nm) on the same cell (Old and Adegbola, 1982). The ultrastructure of *M. morganii* has not been investigated to any extent.

The peptidoglycan of *M. morganii* is O-acetylated at the C-6 hydroxyl group of *N*-acetylmuramyl residues (Clarke, 1993). The degree of O-acetylation ranges from 43.0–49.6% and appears responsible for resistance to muramidase (lysozyme) activity. The major fatty acids present in *M. morganii* lipopolysaccharide are 3-hydroxytetradecanoic ($C_{14:0\ 3OH}$), tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), and dodecanoic ($C_{12:0}$) acids (Vasyurenko and Chernyavskaya, 1990). Cellular fatty acid analysis of morganellae reveals hexadecanoic acid as the dominant peak with octadecenoic acid ($C_{18:1}$) and methylenehexadecanoic acid ($C_{16:0\ ante}$) as additional major peaks (Vasyurenko and Chernyavskaya, 1990). The presence of detectable amounts of dodecanoic acid separates morganellae from *Proteus* and *Providencia*. Histamine, cadaverine, and diaminopropane are the predominant polyamines synthesized under defined conditions (Hamana, 1996).

Half of all morganellae grown in broth culture show uniform turbidity with ring or pellicle formation (McKell and Jones, 1976). Growth occurs between 4 and 45°C. On nutrient agar, *M. morganii* produces smooth transparent colonies with an entire edge. Pigmentation not observed (McKell and Jones, 1976). On nonselective media, most morganellae appear as nonhemolytic, buff-colored, convex colonies, 2–3 mm in diameter after overnight incubation at 35–37°C (Janda and Abbott, 1998a). Hemolysis on blood agar may be detected by prolonged incubation (48–72 h). On media containing an aromatic amino acid such as phenylalanine agar an almond-like odor may be emitted (Müller, 1986a). Because most strains are sucrose- and lactose-negative they appear as colorless colonies on selective media such as MacConkey's, xylose-lysine-desoxycholate, eosin-methylene blue, and Salmonella–Shigella agar. Some strains are highly pleomorphic producing multiple morphovars on media such as Salmonella–Shigella agar (Janda and Abbott, 1998a).

Morganellae possess a number of metabolic features almost exclusively associated with members of the tribe *Proteeae* (*Morganella*, *Proteus*, and *Providencia*). Oxidative deamination of certain amino acids (e.g., phenylalanine deaminase) to form keto acids that then react with ferric compounds yielding chromogenic products is a unique characteristic of this tribe (Singer and Volcani, 1955; Ewing, 1986a). On DL-tryptophan agar, *Morganella* produces a reddish-brown melanin-like compound with a molecular weight ≤12,000 (Polster and Svobodová, 1964; Müller, 1986a). *M. morganii* also degrades L-tyrosine crystals incorporated

into solid media within 24 h, presumably mediated via a tyrosine phenol-lyase (Sheth and Kurup, 1975). All proteeae, including *M. morganii*, produce extracellular bacteriolytic enzymes capable of degrading cell wall components of *Escherichia coli* and *Pseudomonas aeruginosa*. This reaction presumably occurs through extracellular secretion of a peptidoglycan-hydrolase (Branca et al., 1996). Like *Proteus*, morganellae elaborate a type II glycerol dehydrogenase that distinguishes these taxa from *Providencia* (type III); 1,3-propanediol dehydrogenase is not produced (Bouvet et al., 1995a).

The genus is biochemically homogeneous. Indole formed. Nitrates reduced. The methyl red test is positive. The Voges–Proskauer test is negative. Lysine decarboxylase activity is variable. Growth in KCN broth. Citrate, acetate, malonate, and mucate not utilized. Hydrolysis of urea. No H_2S produced on triple sugar iron (TSI) agar slants, although upon prolonged incubation (>24 h) a slight blackening at the junction may occur due to small amounts of a reddish-brown pigment being produced (Janda and Abbott, 1998a).

Acid with gas from D-glucose fermentation. Anaerobic fermentation of D-mannose. Fermentation of glycerol and trehalose is variable (Siboni, 1976). No fermentation of adonitol, L-arabinose, cellobiose, dulcitol, α-methyl-D-glucoside, *m*-inositol, lactose, maltose, D-mannitol, melibiose, raffinose, L-rhamnose, salicin, sucrose, and D-xylose.

Deoxyribonuclease, ribonuclease, alkysulfatase, arylsulfatase, lipase, lecithinase, hyaluronidase, and protease activities not produced. Chitin, elastin, pectin, mucin, and fibrin are not degraded (Janda et al., 1996b). Alkaline and acid phosphatase, leucine arylamidase, and naphthol-AS-BI-phosphohydrolase positive.

Morganella exhibits 20% relatedness to core members of the *Enterobacteriaceae* in DNA hybridization studies (Brenner et al., 1978). All *M. morganii* studied constituted a single DNA relatedness group. The average relatedness of 18 strains was 90% in 60°C reactions and 85% in 75°C reactions with <1% divergence (Brenner et al., 1978). Biochemically aberrant morganellae, exhibiting either the lysine decarboxylase-positive or ornithine decarboxylase-negative phenotype, were subsequently found to be highly related to classic *M. morganii* using the lysine decarboxylase-positive strain, CDC 1274-75, as the source of labeled DNA (Hickman et al., 1980). Hybridization studies indicated 73–92% relatedness in 60°C reactions and 79–96% relatedness in 75°C reactions.

Morganella strains harbor between one and four extrachromosomal elements ranging from 35–60 MDa (Cornelis et al., 1981; Janda et al., 1996b). Plasmids in *Morganella* that confer antibiotic resistance belong to compatibility groups N, FI, and FII (Hedges et al., 1973). Additionally, lactose fermentation and lysine decarboxylation are attributable to plasmid carriage (Le Minor and Coynault, 1976; Cornelis et al., 1981). A bacteriophage typing scheme using seven phages defined 14 lytic patterns, but plaques produced are poorly developed (Schmidt and Jeffries, 1974). Phages are lytic only for *Morganella* and have not been found to attack *Proteus* or *Providencia* (Coetzee, 1963). A bacteriocin (morganocin) system detecting both morganocin production and sensitivity revealed 33 types in 45 serologically distinct strains of *Morganella* (Senior and Vörös, 1989).

The antigenic schema of *Morganella* consists of 77 serotypes with 44 somatic (O), 4 capsular (K), and 38 flagellar (H) antigens (Vörös and Senior, 1990). In 1990, 11 additional O types were added, extending the number of somatic antigens to 55 (Vörös and Senior, 1990). Passive hemagglutination can be used to determine the O antigen (Penner and Hennessy, 1979b).

Morganella is generally resistant to ampicillin, extended-spectrum penicillins, first-generation cephalosporins, and cefoxitin. Antimicrobial agents to which strains are susceptible include third-generation cephalosporins, aztreonam, quinolones, tobramycin, and chloramphenicol. Variable resistance has been demonstrated for gentamicin, amikacin, imipenem, and tetracycline. Disk diffusion testing may be unreliable for some cephems (Biedenbach et al., 1993).

Although isolated more often from urine than other sources, *Morganella* is an uncommon cause of urinary tract infection (UTI) (Janda and Abbott, 1998a). It is often isolated as a colonizer in cases of bacteriuria in patients undergoing long-term catheterization (Mobley and Warren, 1987). Bacteremia is rare, usually occurring in immunocompromised patients; 70% of cases are acquired nosocomially (Janda and Abbott, 1998a). Surgical patients are affected most often, with the focus of infection being wounds. Urinary tract infections are not a common source for bacteremias (McDermott and Mylotte, 1984).

M. morganii produce mannose-sensitive hemagglutinins (MSHA) (Coetzee et al., 1962); electron micrographs of MSHA strains show perifimbriation but with denser polar aggregations. Catecholate- or hydroxymate-type siderophores used for iron acquisition are not produced (Drechsel et al., 1993). However, α-keto acids generated by L-amino acid deaminases produced by *M. morganii* can form ferric complexes that are sufficiently stable to transport iron (Drechsel et al., 1993). *M. morganii* can also use exogenous siderophores (ferrichromes, rhizoferrin, and citrate) for iron acquisition. *M. morganii* strains producing heat labile enterotoxin have been reported in a group of Swedish travelers with diarrhea (Jertborn and Svennerholm, 1991). Approximately 50% of morganellae produce a cell-free hemolysin related to the α-hemolysin of *E. coli* (Koronakis et al., 1987). The cytoplasmic location of urease and its low activity optimum of pH 5.5 in *M. morganii* allows the organism to survive under acidic conditions when other urease-positive, Gram-negative rods die (Young et al., 1996a). However, *M. morganii* grows more slowly and is less efficient in producing alkaline conditions in the urine than *P. mirabilis*, which is a more frequent cause of UTIs (Senior, 1983).

Enrichment and Isolation Procedures

Morganellae may be recovered from specimens from sterile body sites using routine enteric isolation media. Both smooth and rough morphovars may be present. For fecal specimens, MacConkey agar with methyl blue and phenolphthalein diphosphate may be helpful (Janda and Abbott, 1998a). When testing for human intestinal carriage, Rustigian and Stuart (1945) reported increases in *Morganella* recovery of 1.8–10% using tetrathionate or selenite enrichment broth prior to plating on media.

Maintenance Procedures

Morganella strains may be maintained at room temperature in agar deeps, especially motility agar, for several months. For long-term storage cultures should be frozen and maintained at −70°C.

Differentiation of the genus *Morganella* from other genera

See the genus *Proteus*, Table BXII.γ.254, for characteristics that can be used to differentiate *Morganella* from other related genera of *Enterobacteriaceae*.

List of species of the genus Morganella

1. **Morganella morganii** (Winslow, Kliger and Rothberg 1919) Fulton 1943, 81[AL] (*Bacillus morgani* (sic) Winslow, Kliger and Rothberg 1919, 481; *Proteus morganii* (Winslow, Kliger and Rothberg 1919) Yale 1939b, 435.)
mor.ga' ni.i. M.L. gen. n. *morganii* of Morgan; named after H. de R. Morgan, a British bacteriologist who first studied the organism.

The description is the same as that for the genus. Trehalose negative, susceptible to tetracycline See Tables BXII.γ.236 and BXII.γ.237 for other characteristics.

Occurs in the feces of humans, dogs, other mammals, and reptiles. Opportunistic human pathogens.

The mol% G + C of the DNA is: 50 (T_m).

Type strain: ATCC 25830, DSM 30164, IFO 3848, NCIB 235.

a. **Morganella morganii** *subsp.* **morganii** *subsp. nov.* (Winslow, Kliger and Rothberg 1919) Fulton 1943, 81[AL] (*Bacillus morgani* [sic] Winslow, Kliger and Rothberg 1919, 481; *Proteus morganii* (Winslow, Kliger and Rothberg 1919) Yale 1939b, 435.)

Distinguished from subsp. *sibonii* by positive trehalose reaction and resistance to tetracycline. See Table BXII.γ.236 for other characteristics.

The mol% G + C of the DNA is: 50 (T_m).

Type strain: ATCC 25830, DSM 30164, IFO 3848, NCIB 235.

b. **Morganella morganii** *subsp.* **sibonii** *subsp. nov.* Jensen, Frederiksen, Hickman-Brenner, Steigerwalt, Riddle and Brenner 1992, 619[VP]
si.bo' ni.i. L. gen. n. *sibonii* of Siboni, named after Knud Siboni, a Danish microbiologist who first recognized trehalose-fermenting *Morganella morganii*.

Distinguished from subsp. *morganii* by characteristics in Table BXII.γ.236. The above subspecies designations were created in keeping with the recommendation that "genetically close organisms that diverge in phenotype" be given subspecies status; DNA relatedness data for these strains indicated the creation of subspecies as opposed to species.

The mol% G + C of the DNA is: 50.

Type strain: 8103-85, ATCC 49948.

TABLE BXII.γ.236. Characteristics of *Morganella morganii*[a,b]

Characteristic	*Morganella morganii* subsp. *morganii*	*Morganella morganii* subsp. *sibonii*
Indole	+	d
Voges–Proskauer	−	−
Utilization of Simmons citrate, malonate, mucate, acetate	−	−
Urease	+	+
H_2S production (triple sugar iron agar)	d	−
Phenylalanine deaminase	+	+
o-Nitrophenyl-β-galactopyranoside (ONPG) hydrolysis	D	−
Møeller amino acid decarboxylases:		
Lysine decarboxylase	d	d
Arginine dihydrolase	−	−
Ornithine decarboxylase	d	d
Motility	+	d
Gelatin liquefication 22°C	−	−
Growth in potassium cyanide (KCN)	+	d
NO_3^- reduction to NO_2^-	+	+
Corn oil lipase	−	−
Deoxyribonuclease	−	−
Acid production from:		
Glucose, D-mannose	+	+
Trehalose, adonitol, D-arabitol	−	+
L-Arabinose, cellobiose, dulcitol, erythritol, *myo*-inositol, lactose, maltose, mannitol, α-methylglucoside, melibiose, raffinose, rhamnose, salicin, *D*-sorbitol, sucrose, D-xylose	−	−
Tetracycline susceptibility	+	−

[a]For symbols see standard definitions; temperature of reactions, 36 ± 1°C. All reactions are for 48 h.

[b]Adapted from Farmer (1995) and Jensen et al. (1992).

TABLE BXII.γ.237. Identification of *Morganella morganii* biogroups[a,b]

Characteristic	Biogroup						
	A	B	C	D	E	F	G
Acid production from:							
Trehalose	−	−	−	−	+	+	+
Glycerol	+[c]	d	+[c]	−	−	d[c]	d[c]
Møeller amino acid decarboxylase:							
Lysine decarboxylase	−	+	−	+	+	d	−
Ornithine decarboxylase	+	+	−	−	+	−	+
Motility	+[c]	d	d	−	+	d	+
Tetracycline susceptibility	+	+	d	+	−	−	d

[a]For symbols see standard definitions. Temperature of reactions 36 ± 1°C. All reactions are for 48 h.

[b]Adapted from Jensen et al., 1992.

[c]Positive at 3–7 d.

Genus XXII. **Obesumbacterium** *Shimwell 1963, 759*[AL]

J.J. Farmer III and Don J. Brenner

O.be' sum.bac.te' ri.um. L. neut. adj. *obesum* fat; L. neut. n. *bacterium* rod; M.L. neut. n. *Obesumbacterium* a fat, rod-shaped bacterium.

Pleomorphic rods 0.8–2.0 × 1.5–100 μm (short, "fat" rods predominate when grown in beer wort with live yeasts; long pleomorphic rods usually predominate when grown in most bacteriological media; some strains have been reported to display a branching cell morphology), conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. **Nonmotile.** Facultatively anaerobic. Slow growing, forming colonies <0.5 mm in diameter on ordinary plating media at 24 h. Optimal growth temperature is 25–32°C; **growth at 37°C is comparatively poor**. **Acid formed from D-glucose and D-mannose; very few other carbohydrates are fermented.** Gas formation during fermentation appears to be variable (original description says gas is produced, but none of the strains studied produced gas). **Lysine decarboxylase is positive.** Nitrate is reduced to nitrite. **Many biochemical tests normally used for differentiation of *Enterobacteriaceae* are negative or delayed positive** (3–7 d at 36°C). **One of only three genera** (along with *Hafnia* and *Pragia*) **in *Enterobacteriaceae* to contain only gluconate dehydrogenase** in its D-glucose oxidation pathway (Bouvet et al., 1989). **Occurs as a brewery contaminant** that can survive and grow in the presence of live yeasts during beer production. Not isolated from human clinical specimens; no evidence of pathogenicity for humans or animals. The genus has a single species, *O. proteus*, with two defined biogroups (1 and 2). The description of the genus above is a "composite description" based on data for both biogroups. However, the two biogroups are actually distinct species that are phenotypically different and only distantly related by DNA–DNA hybridization (Brenner, 1981). *O. proteus* biogroup 1 is actually a biogroup of *Hafnia alvei* (Brenner, 1981), and will be referred to as *H. alvei* biogroup 1 (Farmer et al., 1985a) in this chapter.

The mol% G + C of the DNA is: 48–49.

Type species: **Obesumbacterium proteus** (Shimwell and Grimes 1936) Shimwell 1963, 759 (*Flavobacterium proteum* (sic) Shimwell and Grimes 1936, 348.)

Further descriptive information

Obesumbacterium, as a member of *Enterobacteriaceae*, belongs to the *Gammaproteobacteria*. Shimwell's original description of an organism he called *"Flavobacterium proteum"* centered on its cellular morphology (Shimwell, 1936; Shimwell and Grimes, 1936). He noted that it appeared as plump rods 0.8–1.2 × 1.5–4 μm when grown in wort media or when taken directly from breweries during fermentation. This morphology had no doubt led to the term "short fat rod of pitching yeasts", which had been used in breweries for many years. Shimwell (1936) also noted much pleomorphism when the organism was grown in laboratory media that were alkaline or neutral. Chains of up to 100 μm were observed under these conditions.

SDS-PAGE protein fingerprints differentiate *H. alvei* biogroup 1 (*O. proteus* biogroup 1) from *O. proteus* biogroup 2 (Fernandez et al., 1993), as do plasmid profiles, ribotyping, enteric repetitive intergenic consensus (ERIC)-PCR (Prest et al., 1994), and random amplified polymorphic DNA (RAPD) profiles (Savard et al., 1994). Plasmid profiling, ERIC-PCR, and RAPD profiles successfully differentiated between isolates of *O. proteus* biogroup 2 (Prest et al., 1994; Savard et al., 1994).

A number of reports discuss the ecology of *Obesumbacterium* in breweries (Shimwell, 1936, 1948, 1963, 1964; Shimwell and Grimes, 1936; Strandskov et al., 1953; Case, 1965). Unfortunately, no distinction has been made between biogroups 1 and 2, so it is usually impossible to determine whether these two biogroups (which are really distinct species, one of which does not belong *Obesumbacterium*; see Taxonomic Comments, below), are different in their ecology, distribution, and other factors. Future studies in breweries should resolve this problem, but only if the two distinct biogroups are differentiated. This approach was used by van Vuuren (1978) in South African breweries.

Changes in brewery practices have eliminated many bacterial contaminants that were once carried along with pitching yeasts; thus *Obesumbacterium* is isolated much less frequently than in the past (F.G. Priest, personal communication). This fact may hamper future studies on the organism.

Enrichment and Isolation Procedures

Quantitative recovery of *O. proteus* from samples of ale yeast was best after 3 d incubation at 25°C on universal beer agar and Wallerstein Laboratories' differential medium, containing cycloheximide to inhibit yeast growth, intermediate on wort agar and yeast mannitol (YM) (Difco) agar, and unsatisfactory on MacConkey agar and membrane lauryl sulfate agar (Fernandez et al., 1993).

Maintenance Procedures

Long-term storage can be accomplished by quick freezing at −80°C in yeast extract peptone dextrose broth supplemented with 15% glycerol (Fernandez et al., 1993), or in 10% skim milk.

Taxonomic Comments

Obesumbacterium was first proposed as a genus by Shimwell in 1963 (Shimwell, 1963, 1964) to accommodate the organism known as *"Flavobacterium proteus"* (Shimwell and Grimes, 1936), which was called *"Flavobacterium proteum"* in the original proposal (Shimwell and Grimes, 1936). The specific epithet *"proteus"* was chosen because the organism has a very pleomorphic cell morphology depending upon the particular growth conditions (Shimwell, 1936, 1948, 1964). The organism was first named in 1936 when Shimwell was doing studies on the "short fat rods of pitching yeasts". He gave an adequate description (based on the available techniques) of the organism, but much of his description is not helpful in identifying it. Shimwell deposited a pure culture of *"F. proteus"* (isolated from yeast of Beamish and Crawford's Brewery, Cork, Ireland) in the National Collection of Type Cultures (NCTC), England. However, when Shimwell's own culture was "lost", he wrote the NCTC and found they had also "lost" this culture (unpublished letter of 27 February 1964 from J.L. Shimwell to E.F. Lessel of the ATCC). Thus it appears that no culture has survived from those originally studied by Shimwell in writing his description of *"F. proteus"*. The strain most studied is apparently ATCC 12841 (NCIB 8771; strain 42 of Strandskov and Bockelmann), which, until 1980 was only a reference strain (*Bergey's Manual of Determinative Bacteriology*, 8th ed.). This strain was isolated from lager and ale yeasts and deposited by the Shaefer

Brewing Co. (Strandskov and Bockelmann, 1955). This strain was given status, without comment, as the type strain (neotype) of *O. proteus* when the Approved Lists of Bacterial Names were issued in 1980.

There is confusion whether the current type strain of *O. proteus* is the same organism that Shimwell studied and named *"F. proteus"*. In 1956, the NCTC sent three cultures (numbers 42, 2, and 41), isolated and described by Strandskov and Bockelmann (1955), to Shimwell to examine and determine whether he thought they were *"F. proteus"*. For no. 42 (the current type strain of *O. proteus*) he concluded: "This is almost certainly an authentic strain. Its morphology is almost exactly that of my original isolation, namely thick (up to 2 or more µm), long (up to 100 µm or more) filaments etc. together with the usual short fat rods in fair numbers". The properties of these strains include indole, acetylmethylcarbinol, H_2S and starch, all negative; nitrite from nitrate, positive. However, probably hundreds of Gram-negative bacteria would answer to this description. Indeed, the species is very poorly characterized biochemically, its main characteristic being its extremely large cell size, and its almost incredible pleomorphism, by means of which (taken in conjunction with its presence in a brewery fermentation) it can readily be identified. In cataloguing any of the strains I suggest that no. 42 could be safely named *F. proteus*; no. 2 a little doubtfully, and no. 41 very doubtful indeed" (unpublished letter of 7 June 1956 from J.H. Shimwell to W.S. Greaves of the NCTC). Thus the current concept of *O. proteus* based on its type strain ATCC 12841 (no. 42) is not incompatible with *"F. proteus"* as defined by Shimwell based on strains that no longer exist. Unfortunately, Shimwell' s original description of *"F. proteum"* fits both *H. alvei* biogroup 1 (*O. proteus* biogroup 1) and *O. proteus* biogroup 2, so it is uncertain which of these latter two organisms (or perhaps both) was originally studied by Shimwell (1936). It is even possible that it was neither since Shimwell described *"F. proteum"* as a producer of gas during fermentation of carbohydrates, but neither *H. alvei* biogroup 1 nor *O. proteus* biogroup 2 produce gas (Priest et al., 1973; see also Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*.

"F. proteus" had been known for many years as a brewery contaminant that can survive and grow in the presence of live yeasts during beer production. Because it fermented D-glucose and other carbohydrates, it was incompatible with a redefined genus *Flavobacterium* that was limited to oxidative rather than fermentative bacteria. Its removal from *Flavobacterium* was subsequently confirmed by Bauwens and De Ley (1981), who used DNA–rRNA hybridization to show that *"F. proteus"* was not closely related to other *Flavobacterium* species. Shimwell (1963, 1964) formed the new genus *Obesumbacterium* for *"F. proteus"* because its phenotypic properties and ecological niche differed from those of other genera. He did not assign *Obesumbacterium* to a family, but its properties (see also Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*) are compatible with those of the family *Enterobacteriaceae*.

Priest et al. (1973) determined the phenotypic properties and did DNA–DNA relatedness studies on 19 strains of *O. proteus*, including 16 brewery isolates. They defined two biogroups, which had the same mol% G + C content of DNA, 48.0–48.5. They also proposed that *O. proteus* be reclassified in the genus *Hafnia* where its citation would be *"H. protea"* (Shimwell and Grimes, 1936) Priest, Somerville, Cole and Hough 1973. The new combination could have been proposed as *"Hafnia proteus"*, since *"proteus"* is a substantive that need not agree in gender with its genus. This change in classification was accepted to some extent, but in reality both *O. proteus* and *"H. protea"* have, until recently, been rarely used in the literature. Most of the existing citations have been in journals related to brewing. The name *"Hafnia protea"* lost standing in nomenclature on 1 January 1980, because it did not appear on the Approved Lists of Bacterial Names; however, *Obesumbacterium* and *O. proteus*, with its type strain ATCC 12841, have standing in nomenclature since they did appear on the lists.

The classification of *Obesumbacterium* was clarified by Brenner and co-workers (1981), who used DNA–DNA hybridization to determine the relatedness of *O. proteus* biogroups 1 and 2 to each other and to other *Enterobacteriaceae*. *O. proteus* biogroup 1 was very highly related to *Hafnia alvei*, and it was concluded that this biogroup is a synonym of *H. alvei*. It can best be thought of as the pleomorphic, KCN-negative, nonmotile, non-gas-producing, salicin-positive, L-arabinose-negative, L-rhamnose-negative, maltose-negative, D-xylose-negative, β-galactosidase-negative biogroup of *H. alvei* that has adapted to the brewery environment. This adaptation to the brewery environment was noted by Shimwell and Grimes (1936) in the original description of *"F. proteus"*: "The organism sometimes failed to grow in dilute media, probably owing to its having become accustomed to the more concentrated nature of beer-wort in the brewery." This adaptation has presumably made the organism very "sluggish" in its metabolic activities, as is reflected in its slow growth rate and diminished activity in the tests normally done for identification of *Enterobacteriaceae*. The classification of *O. proteus* biogroup 1 as *H. alvei* is further strengthened by the fact that strains of *O. proteus* biogroup 1 (but not biogroup 2) are lysed (Farmer, 1984a; Table BXII.γ.238) by the *Hafnia*-specific bacteriophage 1672 described by Guinée and Valkenburg (1968).

Van Vuuren et al. (1981) studied 10 cultures of "*Hafnia alvei*-*Obesumbacterium proteus*" isolated from South African lager-beer breweries. One of their five distinct biogroups (based on API 20E biochemical profiles) was very active biochemically and more like typical cultures of *H. alvei*. The other four biogroups were progressively less biochemically active with the least active group more like *O. proteus*. Based on phenotypic and DNA relatedness data (Brenner, 1981), Farmer et al. (1985a) proposed that *O. proteus* biogroup 1 and biochemically inactive strains of *H. alvei* isolated from breweries be classified in a single taxon. They proposed the names " *Hafnia alvei* biogroup 1" and " *Hafnia alvei* brewery biogroups" to aid in the recognition and identification of these strains adapted to the brewery environment. *H. alvei*, *H. alvei* biogroup 1, and *O. proteus* biogroup 2 have been listed as separate taxa in the CDC' s master biochemical chart of *Enterobacteriaceae* since 1985. Based on these data, it appears that biogroups intermediate between typical *H. alvei* and *O. proteus* biogroup 1 may occur in breweries. A complete set of biochemical tests (Table BXII.γ.238, also see Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*) and lysis by the *Hafnia*-specific bacteriophage are needed to characterize isolates from breweries that resemble *Hafnia/ Obesumbacterium*.

O. proteus biogroup 2 is different biochemically from *H. alvei* biogroup 1 and from *H. alvei* (Table BXII.γ.238). In DNA–DNA relatedness studies *O. proteus* biogroup 2 was only 25–30% related to *H. alvei* biogroup 1 (Brenner, 1981). In fact, its closest relative is *Escherichia blattae* to which it was 60–65% related. Thus *O. proteus*

TABLE BXII.γ.238. Differentiation of *Obesumbacterium proteus* biogroup (BG) 2, *Hafnia alvei*, and *Hafnia alvei* biogroup 1 (*Obesumbacterium proteus* biogroup 1)[a]

Characteristic	Incubation time (d)	*O. proteus* BG 2	*Hafnia alvei* wildtype	*Hafnia alvei* BG 1 [b]
Lysis by the *Hafnia*-specific bacteriophage of Guinée and Valkenburg (1968)	1	−	+	+
Strong rapid catalase[c]		−	+	+
Voges–Proskauer (22°C)	4	−	+	+
Acid production from:				
D-Mannitol	10	−	+	+
Salicin	7	−	[−]	+
D-Xylose	7	+	+	−
Esculin hydrolysis	7	−	[−]	+

[a]Symbols: +, positive for 90–100% of strains; [−], positive for 11–25% of strains; −, positive for 0–10% of strains.

[b]Formerly known as *O. proteus* biogroup 1.

[c]Strains of *O. proteus* biogroup 2 are catalase positive but the reaction is weak and takes 10–30 seconds. Strains of wild-type *H. alvei* and of *H. alvei* biogroup 1 give a very strong and rapid (1 second) catalase reaction. This is a very simple way to differentiate between strains of *H. alvei* and *O. proteus* biogroup 2.

biogroup 2 should not be included in the same species or in the same genus as *H. alvei* biogroup 1.

The type strain of *O. proteus* is ATCC 12841 (NCIB 8771). It was listed as *"H. protea"* in the 14th edition of the ATCC catalog, and has been listed as *O. proteus* in the 15th to 19th editions. This culture is lysed by the *Hafnia*-specific bacteriophage 1672, is methyl red negative, D-mannitol negative, salicin positive (6 d), maltose negative, D-xylose negative, esculin positive (7 d), and ONPG negative. Thus from Table BXII.γ.238 it clearly belongs to *H. alvei* biogroup 1, although it is somewhat more inactive or slower in its biochemical reactions than other strains. *H. alvei* biogroup 1 can be logically considered as a series of inactive biogroups of *H. alvei*. However, if it were classified as a named subspecies it could cause serious nomenclatural problems in *Hafnia* because it has priority over *H. alvei*. Since the name *Obesumbacterium* has traditionally been well known in the brewing industry, which seems to be the ecological niche for this organism, the most practical solution at present is to continue use of the names *H. alvei* biogroup 1 and *O. proteus* biogroup 2 until all the taxonomic problems are resolved and a "final" nomenclature and classification can be proposed after careful analysis.

Since *O. proteus* is now known to be a heterogeneous species, it is essential to append "biogroup 2" to the name that will correspond to the second distinct species shown by DNA–DNA hybridization and biochemical tests. Unless this is done, the intended meaning of *O. proteus* is unclear. We recommend that the term *O. proteus* biogroup 1 be discontinued, and replaced by *H. alvei* biogroup 1.

O. proteus biogroup 2 is a unique genomospecies (DNA hybridization group); however, since its type strain is in a different hybridization group than that of *H. alvei*, the species and the genus (since it is the type species of the genus) would be invalid because it is a junior synonym of *H. alvei*. To rectify this, a new type strain would have to be designated. A Request for an Opinion to do this would have to be made to the Judicial Commission. If the request were granted, the genus *Obesumbacterium* and the species *O. proteus* would again be valid under the rules of nomenclature of the Bacteriologic Code. Other alternatives remain since, based on its DNA–DNA relatedness to *Escherichia blattae*, *O. proteus* could be classified in the genus *Escherichia*. Placing it in this genus would be a simple matter—designating a new type strain and describing a new combination: *"Escherichia proteus"*. An alternative would be to classify *E. blattae* and *O. proteus* biogroup 2 together, either in a redefined genus *Obesumbacterium* or as a new genus. The results from 16S rDNA sequencing (not currently available), should be helpful in deciding on the most logical classification.

Further Reading

Case, A.C. 1965. Conditions controlling *Flavobacterium proteus* in brewery fermentations. J. Inst. Brew. *71*: 250–256.

Priest, F.G., H.J. Somerville, J.A. Cole and J.S. Hough. 1973. The taxonomic position of *Obesumbacterium proteus*, a common brewery contaminant. J. Gen. Microbiol. *75*: 295–307.

Shimwell, J.L. 1936. A study of the common rod bacteria of brewers' yeast. J. Inst. Brew. *42*: 119–127.

List of species of the genus Obesumbacterium

1. **Obesumbacterium proteus** (Shimwell and Grimes 1936) Shimwell 1963, 759[AL] (*Flavobacterium proteum* (sic) Shimwell and Grimes 1936, 348.)

   pro' te.us. Gr. masc. n. *proteus* the ancient Greek sea-god noted for being able to change his form at will; Gr. masc. n. *proteus* pleomorphic.

   Obesumbacterium proteus is really two different species, which can be differentiated by phenotypic tests (Table BXII.γ.238) and DNA–DNA relatedness tests. The type strain has the properties of *H. alvei* (*O. proteus*) biogroup 1 (see Taxonomic Comments, above). The description of *O. proteus* biogroup 2 is as given for the genus and as listed in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Occurs in breweries where it grows in beer-wort along with yeasts early in the fermentation. There is no evidence that it is pathogenic for humans or animals.

The mol% G + C of the DNA is: 48–49 (Bd).

Type strain: 42 of Strandskov and Bockelmann (1955), ATCC 12841, DSM 2777, NCIB 8771.

GenBank accession number (16S rRNA): AJ233422.

Additional Remarks: Strain No. 42 belongs to *H. alvei* (O. proteus) biogroup 1. Therefore, at present there is no valid type strain for *O. proteus* biogroup 2—the true representative of the species. Strains 520, 531, and 580 of Priest et al. (1973) belong to *O. proteus* biogroup 2 by DNA–DNA hybridization and are phenotypically typical (Table BXII.γ.238). Thus, they should be considered as the candidates for the proposed type strain.

Genus XXIII. **Pantoea** *Gavini, Mergaert, Beji, Mielcarek, Izard, Kersters and De Ley 1989b, 343^{VP} emend. Mergaert, Verdonck and Kersters 1993, 171*

PATRICK A.D. GRIMONT AND FRANCINE GRIMONT

Pan.toe'a. Gr. adj. *pantoios* of all sorts and sources; M.L. fem. n. *Pantoea* [bacteria] from diverse [geographical and ecological] sources.

Straight rods, 0.5–1.3 × 1.0–3.0 μm. Nonencapsulated. Nonsporeforming. Some strains form symplasmata. **Most strains are motile and are peritrichously flagellated.** Gram negative. Colonies on nutrient agar are smooth, translucent, and more or less convex with entire margins or heterogenous in consistency and adhering to the agar. **Colonies are yellow, pale beige to pale reddish yellow, or nonpigmented. Facultatively anaerobic. Oxidase negative. Glucose dehydrogenase and gluconate dehydrogenase are produced** and are active without an added cofactor. Acid is produced from the fermentation of D-fructose, D-galactose, trehalose, and D-ribose. Most strains are **Voges–Proskauer positive. Lysine and ornithine are not decarboxylated.** Urease negative. Pectate is not degraded. H_2S is not produced from thiosulfate. Optimum temperature 30°C. *N*-acetyl-D-glucosamine, L-aspartate, D-fructose, D-galactose, D-gluconate, D-glucosamine, D-glucose, L-glutamate, glycerol, D-mannose, D-ribose, and D-trehalose are utilized as sole sources of carbon and energy. 5-Aminovalerate, benzoate, caprate, caprylate, *m*-coumarate, ethanolamine, gentisate, glutarate, histamine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, maltitol, D-melezitose, 1-*O*-methyl-α-D-glucoside, palatinose, 3-phenylpropionate, propionate, L-sorbose, tricarballylate, tryptamine, D-turanose, and L-tyrosine are not utilized as sole sources of carbon and energy. *Pantoea* spp. are isolated from plants, seeds, fruits, soils, water, and from humans (urine, blood, wounds, internal organs), and animals. Some strains are (or have been thought to be) phytopathogenic.

The mol% G + C of the DNA is: 49.7–60.6.

Type species: **Pantoea agglomerans** (Ewing and Fife 1972) Gavini Mergaert, Beji, Mielcarek, Izard, Kersters and De Ley 1989b, 343 (*Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972, 10.)

FURTHER DESCRIPTIVE INFORMATION

Much of our knowledge about the characteristics of the genus *Pantoea* was acquired when all *Pantoea*, *Leclercia* and related bacteria were called *Enterobacter agglomerans*. Therefore, in this chapter, we will refer to *Pantoea agglomerans* when this precise species is meant and to the *Enterobacter agglomerans* complex when there is insufficient taxonomic information. Older data refer to *Erwinia herbicola* or the "herbicola group". Strains in the *Enterobacter agglomerans* complex (or *Erwinia herbicola* or the "herbicola group") may or may not belong to the genus *Pantoea*.

Phylogenetic treatment Of the 124 strains of the *Enterobacter agglomerans* complex analyzed by DNA–DNA hybridization by Brenner et al. (1984a), 90 formed 13 distinct DNA groups. The remaining 34 strains did not fit any of these groups. The synonymy between these groups and named species is given in Table BXII.γ.239.

Comparison of *rrs* gene (encoding 16S rRNA) sequences showed the genus *Pantoea* (represented by *Pantoea agglomerans*, *Pantoea ananatis*, and *Pantoea stewartii*) to constitute a monophyletic cluster distinct from clusters corresponding to the genera *Erwinia*, *Pectobacterium*, *Brenneria*, and other genera of the *Enterobacteriaceae* (Hauben et al., 1998a). When all DNA groups from Brenner et al. (1984a) and named *Pantoea* species are studied

TABLE BXII.γ.239. Correspondence between DNA relatedness groups, phenons, and nomenspecies in the *Enterobacter agglomerans* complex.

Gavini et al., 1983b	Brenner et al., 1984a	Verdonck et al., 1987	Nomenspecies (older synonyms)
phenon	DNA group	phenon	
–	I	–	*Pantoea* species
–	IV	–	*Pantoea* species
(B4)[a]	V	7B	*Pantoea* species
	XIII	8	*P. agglomerans* (*Enterobacter agglomerans*, *Erwinia herbicola*, *Erwinia milletiae*)
(B4)			
(B5)	II	9	*Pantoea* species
(B5)	III	10	*Pantoea dispersa*
(B9)	–	11	–
	X	ungrouped	Close to the genus *Buttiauxella*
–			
	VI	12	*Pantoea ananatis* (*Erwinia ananas*, *Erwinia uredovora*)
B8			
	XII	16	Close to the *Enterobacter cloacae* complex
ungrouped			
C	–	(17)	*Rahnella aquatilis*
D1	–	(17)	Close to *Rahnella aquatilis*
ungrouped	IX	18	*Enterobacter cowanii*
	VIII	23	Close to *Enterobacter persicina* and *Erwinia rhapontici*
(B9)			
E2, E3, E5	XI	(26)	*Leclercia adecarboxylata*
	VII	(26)	Close to *Leclercia adecarboxylata* and *Enterobacter cloacae*
E4			

[a] ( ) : parentheses indicate partial correspondence.

by *rrs* and *rpoB* sequence comparisons, the following congruent clusterings are obtained:

a. *P. agglomerans* (DNA group XIII), *P. ananatis* (DNA group VI), *P. stewartii*, and DNA group V cluster together.
b. DNA groups I and II and a cluster containing *Pantoea dispersa* (DNA group III) and DNA group IV branch with the above-mentioned cluster to constitute a monophyletic *Pantoea* cluster.
c. *Pantoea citrea*, *Pantoea terrea*, and *Pantoea punctata* constitute a discrete cluster that joins the *Pantoea* cluster at a lower level.
d. *E. agglomerans* DNA groups VII, VIII, IX, X, XI, and XII branch away from the *Pantoea* cluster (unpublished data).

More taxonomic work is needed to justify the assignment of *P. citrea*, *P. terrea*, and *P. punctata* to the genus *Pantoea*.

Cell morphology Symplasmata are sausage-shaped zoogloeal masses of bacteria observed by phase contrast microscopy in hanging drop preparations from the water of syneresis in glucose nutrient agar slopes after 24 h incubation (Graham and Hodgkiss, 1967). Symplasmata have been observed in bacteria now reclassified in the genus *Pantoea* (*"Bacterium herbicola"*, *Erwinia lathyri*, *Erwinia uredovora*, and *Erwinia milletiae*) (Graham and Hodgkiss, 1967). The epithet *"agglomerans"* probably refers to symplasmata (Beijerinck, 1888).

Nutrition and growth conditions Best results are obtained when *Pantoea* cultures are incubated at 30°C. Most strains grow at 37°C on plating media, but some cultures fail to grow or only produce microcolonies. A few isolates can grow on brilliant green or bismuth sulfite agar media and can produce light growth of colonies that range from very small to 2.0 mm in diameter. Many strains produce mucoid colonies at 37°C, and most cultures do so when the media are incubated at room temperature, about 25°C.

On nutrient agar, anaerogenic strains of the *E. agglomerans* complex (*Pantoea* spp.) form mucoid colonies, smooth and irregularly round colonies, or rough and wrinkled colonies that are difficult to remove with a platinum wire. This latter aspect is what led to the description of "biconvex bodies", which can be observed with a low power stereoscopic binocular microscope in 2 or 3 day-old colonies. These bodies appear to be granular structures analogous to symplasmata or down-growths of the colonies into the medium (Graham and Hodgkiss, 1967).

P. agglomerans, *P. stewartii*, and *P. ananatis* colonies are usually yellow pigmented; *P. dispersa* is often pigmented; and *P. citrea*, *P. punctata*, and *P. terrea* are never pigmented. Yellow pigmentation of *P. agglomerans* may be weak at 37°C.

In Biotype-100 strips (BioMerieux, Craponne, France) or in a minimal medium containing ammonium sulfate as the nitrogen source, the following compounds serve as sole carbon sources for most strains: *N*-acetyl-D-glucosamine, L-aspartate, D-fructose, D-galactose, D-gluconate, D-glucosamine, D-glucose, L-glutamate, glycerol, D-mannose, D-ribose, and D-trehalose.

Most strains cannot utilize the following substrates as sole carbon and energy sources: 5-aminovalerate, benzoate, caprate, caprylate, *m*-coumarate, ethanolamine, gentisate, glutarate, histamine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, maltitol, D-melezitose, 1-*O*-methyl-α-D-glucoside, palatinose, 3-phenylpropionate, propionate, L-sorbose, tricarballylate, tryptamine, D-turanose, and L-tyrosine.

Metabolism Most cultures produce acid from sucrose and therefore yield acid throughout in tubes of triple sugar iron agar. *P. agglomerans* gives negative reactions in all three decarboxylase tests.

Under aerobic conditions, all *Pantoea* species produce gluconate from D-glucose in the presence of iodoacetate due to a glucose dehydrogenase, without added pyrroloquinoline quinone (PQQ) (Bouvet et al., 1989). A reducing compound, 2-ketogluconate, is also produced from gluconate due to a gluconate dehydrogenase (Bouvet et al., 1989). 2,5-Diketogluconate is produced from 2-ketogluconate by *Pantoea citrea*, *P. punctata*, and *P. terrea* (Kageyama et al., 1992).

Pantoea species are negative for the following tests: H_2S production from thiosulfate, tetrathionate reduction, tributyrin and corn oil hydrolysis, and β-glucuronidase.

Mutants, plasmids, phages and phage typing, bacteriocins *Pantoea agglomerans* strain Eh318 produces two antibiotics (pantocin A and B) that inhibit *Erwinia amylovora in vitro* (Wright et al., 2001). *Erwinia herbicola* produces two lipopeptide antibiotics, herbicolicin A and B, which are active against sterol-containing fungi (Greiner and Winkelmann, 1991). Four antibiotics, agglomerins A, B, C, and D, were isolated from the culture broth of a strain identified as *Enterobacter agglomerans*; these antibiotics are active against a wide variety of anaerobic bacteria *in vitro* (Shoji et al., 1989).

The eight bacteriocin-producing *Enterobacter cloacae* strains used to type *E. cloacae* isolates by bacteriocin susceptibility are useful in typing isolates identified as *Enterobacter agglomerans* (Bauernfeind and Petermüller, 1984).

Antigenic structure The principal antigens in most strains studied under the name *E. agglomerans* (*Erwinia herbicola*) are uncharged capsular polysaccharides. These antigens are not removed from the cells by heating at 100°C for 30 min (Slade and Tiffin, 1984). Most members of the "herbicola group" are motile, but there is no report on the antigenic structure of the flagella (Slade and Tiffin, 1984). Most strains produce antigenically similar high molecular weight acidic polysaccharides.

Two schemes of serotyping have been proposed for *Erwinia herbicola*. Muraschi et al. (1965) used immunodiffusion and antigens extracted by aqueous ether and classified 55 isolates into 7 serotypes, although "some cultures were mixtures of more than one serotype". A second scheme was established by Slade (cited by Slade and Tiffin, 1984), using laboratory strains. This scheme has not been used to type new isolates.

Pathogenicity Strains of the *E. agglomerans* complex may occur in clinical samples (blood, wounds, sputum, urine), often with dubious clinical significance (von Graevenitz, 1970; Gilardi and Bottone, 1971; Pien et al., 1972). In some cases (Cooper-Smith and von Graevenitz, 1978; von Graevenitz and Palermo, 1980), clinical significance has been demonstrated. In an Ohio hospital, *E. agglomerans* accounted for four of 58 episodes of *Enterobacter* bacteremia and one of 42 cases of nosocomial bacteremia (Watanakunakorn and Weber, 1989). In 1970, *E. agglomerans* was implicated in a United States-wide and a Canadian outbreak of septicemia caused by contaminated closures on bottles of infusion fluids. Twenty-five hospitals were involved with 378 cases and 40 deaths (Maki et al., 1976).

Strains identified as *Pantoea agglomerans* have been isolated from joint fluid of patients with arthritis following injuries with plant thorns, wood slivers, or wooden splinters (Flatauer and Khan, 1978; Olenginski et al., 1991; de Champs et al., 2000).

A case of metastatic endophthalmitis caused by a strain identified as *Enterobacter agglomerans* was reported. One day following

internal hemorrhoidal ligation, the patient developed anterior uveitis, followed by panophthalmitis and loss of vision (Zeiter et al., 1989).

Enterobacter agglomerans was isolated from the blood of a patient with cotton fever, a benign febrile leukocytic syndrome seen in intravenous narcotic abusers. The strain was also isolated from cotton that the patient had used to filter heroin (Ferguson et al., 1993). *Enterobacter agglomerans* is known to heavily colonize cotton and cotton plants.

Antibiotic sensitivity Most *E. agglomerans* strains are naturally resistant to ampicillin and cephalothin and susceptible to many antibiotics including aminoglycosides, carbenicillin, cefamandole, cefuroxime, and cefoxitine. Resistance to carbenicillin may occur.

Pathogenesis Contaminated medicinal agents can be sources of outbreaks. Maki and Martin (1975) showed that strains identified as *E. agglomerans* (as well as strains of *E. cloacae* and *Serratia marcescens*) multiplied in 5% dextrose solution at 25°C better than did other members of the *Enterobacteriaceae*. A single tested strain of *E. agglomerans* was devoid of hemagglutinin (Adegbola and Old, 1983a). *E. agglomerans* strains produce aerobactin. Two of nine strains of *E. agglomerans* produced a hydroxamate compound other than aerobactin (Reissbrodt and Rabsch, 1988). In another study, strains identified as *Enterobacter agglomerans* produced hydroxamate siderophores identified as ferrioxamine E. The strains had also multiple siderophore receptors in the outer membrane (Berner et al., 1988).

The lipopolysaccharide from strains identified as *E. agglomerans* (commonly found in cotton dust) can bind to the pulmonary lipid-proteinaceous lining material (surfactant) and alter its surface tension properties (DeLucca et al., 1988). This binding in the lung may change the physiological properties of surfactant and be a possible mechanism for the pathogenesis of byssinosis, an occupational respiratory disorder caused by the inhalation of cotton dust (DeLucca et al., 1988).

Ecology The *Enterobacter agglomerans* complex is ubiquitous in the environment. This complex predominates on the leaf and bract of pre- and postsenescent cotton plants (DeLucca and Palmgren, 1986). The main species of Gram-negative bacteria in cotton dusts found in mills is *E. agglomerans* (Haglind et al., 1981). The following occurrences have been reviewed by Slade and Tiffin (1984). Strains of the *Enterobacter agglomerans* complex are found on the aerial surfaces of plants and within healthy plant tissues and seeds. Nitrogen fixing strains have been found in the rhizosphere of wheat and sorghum. In fact, these bacteria are typical of the innermost part of the rhizosphere of wheat (Kleeberger et al., 1983). Nitrogen-fixing strains identified as *Enterobacter agglomerans* have also been isolated from gut of the wood-eating termite *Coptotermes formosanus* (Potrikus and Breznak, 1977). Strains of this complex have been isolated from water, paper mill process water, soil and decaying wood. They are frequently isolated from damaged plant tissues and lesions, although they are rarely considered pathogenic. A pathogenic role has been shown in some instances. Strains named *Erwinia milletiae* produce β-indolyl acetic acid (a plant hormone) causing galls on some plants in Japan (reviewed by Slade and Tiffin, 1984). Some strains initiate freezing of buffer solutions (which would normally freeze at temperatures below −10°C) at about −4°C. This ice-nucleating property plays a critical role in causing frost damage on plants (Kozloff et al., 1983; Lindow et al., 1978).

Pink disease of pineapple, caused by *Pantoea citrea*, is characterized by a dark coloration on fruit slices after autoclaving (Cho et al., 1980). This coloration is initiated by the oxidation of glucose to gluconate, then the oxidation of gluconate to 2-ketogluconate, and finally the production of 2,5-diketogluconate. The latter appears to be responsible for the dark color characteristic of the pink disease of pineapple (Pujol and Kado, 2000).

Some strains of the *Enterobacter agglomerans* complex are associated with stalk and leaf necrosis of onion (Hattingh and Walters, 1981). Strains originally named *Erwinia uredovora* (now *Pantoea ananatis*) attack the uredia of rust (*Puccinia* spp.) on wheat, oats and rye (Pon et al., 1954; Dye, 1969b).

Stewart's bacterial wilt is a disease of corn (*Zea mays*) caused by *Pantoea stewartii* subsp. *stewartii*. On corn seedlings at the three- to five-leaf stage, the disease is characterized by water-soaked lesions on leaves, leading to stunted plants with severe yield reductions in susceptible corn hybrids (Dillard and Kline, 1989; Wilson et al., 1994). The corn flea beetle, *Chaetocnema pulicaria*, is the overwintering host and vector of *P. stewartii*, and the abundance of the primary inoculum is related to corn flea beetle populations. Many countries ban import of seed corn unless it is certified free of *P. stewartii*. A ligase chain reaction assay has been proposed for the detection of *P. stewartii* in infected plant and vector material (Wilson et al., 1994).

P. stewartii subsp. *stewartii* produces an extracellular heteropolysaccharide capsule, which plays several roles in the development of Stewart's wilt on sweet corn. The capsule provides a barrier that protects the bacterium against plant host defense factors (Braun, 1982; Beck von Bodman and Farrand, 1995). It also partially contributes to the induction of water-soaking symptoms early in the development of Stewart's wilt, and it obstructs the free flow of water in the host vascular system, causing necrosis and wilting during the systemic phase of the infection (Braun, 1982; Beck von Bodman and Farrand, 1995). *P. stewartii* subsp. *stewartii* synthesizes *N*-(-3-oxohexanoyl)-L-homoserine lactone, which is an autoinducer for capsular polysaccharide biosynthesis and *P. stewartii* pathogenicity (Beck von Bodman and Farrand, 1995).

A strain identified as *Pantoea agglomerans* was isolated from necrotic spots in the leaves of a beach pea (*Lathyrus maritimus*) that grew on the shorelines of Newfoundland, Canada. The bacterium produced cellulase and amylase and was a plant wound parasite (Khetmalas et al., 1996).

Some strains of the *E. agglomerans* complex have been used for biological control of plant pathogens (such as *Erwinia amylovora* or *Xanthomonas oryzae*), either by competition for nutrients, acid production, bacteriocin production or phage production (reviewed by Slade and Tiffin, 1984). *Pantoea agglomerans* CFA-2 is effective for the biological control of postharvest pear diseases due to *Botrytis cinerea*, *Penicillium expansum*, and *Rhizopus stolonifer* (Nunes et al., 2001).

Pantoea agglomerans in fecal pellets of locusts produces large amounts of guaiacol and small amounts of phenol, both of which are components of the locust cohesion pheromone (Dillon et al., 2002).

Enrichment and Isolation Procedures

All media designed for the isolation of *Enterobacteriaceae* can be used for the isolation of *Pantoea* species: MacConkey agar, Drigalski lactose agar, Hektoen agar, deoxycholate lactose citrate agar, etc. *Pantoea* strains can also grow on media for general use, such as blood agar, nutrient agar, tryptic soy agar, bromocresol

purple lactose agar, etc. Media specifically selective for *Pantoea* strains are not available.

A differential medium, lysine-ornithine-mannitol agar containing vancomycin, was proposed for the isolation of *Pantoea agglomerans*. It yields colorless colonies from mannitol-negative strains, yellow colonies from mannitol-positive, ornithine- and lysine-decarboxylase negative strains, and greenish-blue colonies from mannitol-positive strains that produce one or both decarboxylases (Bucher and von Graevenitz, 1982).

Maintenance Procedures

Strains are initially grown on tryptic soy agar at their optimum temperature. They are then inoculated by stabbing a maintenance medium[1] designed for maintenance of *Enterobacteriaceae* and related organisms. The cultures are then stored at room temperature in a dark, dry place.

Cultures may be also preserved by freeze-drying. Freeze-drying is the best procedure for preservation of pigmented strains.

Differentiation of the genus *Pantoea* from other genera

Enterobacter strains that are negative for lysine and ornithine decarboxylases and arginine dihydrolase and that may or may not produce a yellow pigment were formerly identified as *Enterobacter agglomerans*. The genus *Pantoea* is a subset of the *Enterobacter agglomerans* complex. Species of *Pantoea* are able to oxidize D-glucose to D-gluconate (glucose dehydrogenase activity) without added pyrroloquinoline quinone, and D-gluconate to 2-ketogluconate (gluconate dehydrogenase activity). In addition, they cannot utilize phenylacetate, and most strains can utilize *myo*-inositol.

Taxonomic Comments

The genus *Erwinia* has long been a depository for plant-associated members of the family *Enterobacteriaceae*. Several species of this genus were found to be phenotypically similar to *Enterobacter* species (*Erwinia herbicola*, *Erwinia ananatis*, *Erwinia uredovora*, *Erwinia milletiae*, *Erwinia dissolvens*, and *Erwinia nimipressuralis*) (Lelliott, 1974; Lelliott and Dickey, 1984), or highly related to *Enterobacter* species by DNA hybridization (*Erwinia dissolvens* and *Erwinia nimipressuralis*) (Steigerwalt et al., 1976).

The (invalid) name *"Bacterium herbicola aureum"* was introduced by Düggeli (1904) and *"Bacterium herbicola"* is attributed to Löhnis (1911). The species was transferred to the genus *Erwinia* as *E. herbicola* by Dye (1964). It has always been considered a saprophyte associated with plants (epiphyte).

The earliest synonym of *Erwinia herbicola*, represented by an extant culture, is *"Pseudomonas trifolii"* (Hüss 1907) shown by Dye (1964) to be identical with *Erwinia herbicola*. Unfortunately, the epithet *"trifolii"* did not appear in the Approved Lists, although it had priority over the epithet *herbicola*.

Manns and Taubenhaus (1913) described *"Bacillus lathyr"* as the cause of streak disease of sweet peas (now attributed to a virus). The species was transferred to the genus *Erwinia* as *E. lathyri* by Holland (1920).

"Bacillus milletiae" was described by Kawakama and Yoshida (1920) as causing galls on millet. The species was transferred to the genus *Erwinia* as *E. milletiae* by Magrou (1937).

"Bacillus ananas" was isolated from bacterial fruitlet brown-rot of pineapple in the Philippines by Serrano (1928). It is given as *Erwinia ananas* Serrano 1928 on the Approved Lists of Bacterial Names (Skerman et al., 1989).

"Xanthomonas uredovorus" was isolated from uredia of cereal rusts (Pon et al., 1954) and reclassified in the genus *Erwinia* by Dye (1963c).

Graham (1958) reported that the pathogenic properties of *E. ananas* and *E. milletiae* are doubtful and that culturally and biochemically these species were indistinguishable from *E. lathyri*.

Dye (1969b) proposed a classification of the "herbicola group" containing *Erwinia herbicola* subsp. *herbicola* (18 species names were listed as synonyms including *E. herbicola*, *E. lathyri*, and *E. milletiae*), *Erwinia herbicola* subsp. *ananas*, *E. uredovora* and *E. stewartii*.

"Pseudomonas stewartii" was isolated by Smith (1898) from Stewart's sweet corn wilt and was transferred to *Erwinia* as *E. stewartii* by Dye (1963b).

After studying many cultures of the "herbicola-lathyri" group, including clinical isolates, Ewing and Fife (1972) exhumed an old work by Beijerinck (1888) describing *"Bacillus agglomerans"*, a bacterium that formed what might have been symplasmata. Although no culture was available, a new combination, *Enterobacter agglomerans* (Beijerinck) Ewing and Fife 1972, was proposed for the "herbicola-lathyri" group on the argument of priority. However, the Approved Lists gave the name as *Enterobacter agglomerans* Ewing and Fife 1972, thus losing the connection with Beijerinck (1888). The other synonyms found in the Approved Lists are *Erwinia herbicola* (Löhnis 1911) Dye 1964 and *Erwinia milletiae* (Kawakama and Yoshida 1920) Magrou 1937.

E. agglomerans is a very complex group of bacteria, which may cause opportunistic infections. The name covers many (20 to 40) genomic groups (Brenner et al., 1984a) or phena (Gavini et al., 1983b; Verdonck et al., 1987). In addition to this diversity, strains of the *E. agglomerans* complex are not closely related to *E. cloacae* (the type species of the genus *Enterobacter*) by DNA relatedness. Some groups in this complex have been designated as new genera (*Rahnella aquatilis*, *Ewingella americana*, *Leclercia adecarboxylata*). There is an apparent confusion in the literature, and a close examination of published papers for the presence of commonly studied strains is needed to extract convergent pieces of information. A table relating phena and DNA groups has been created after such a literature analysis (Table BXII.γ.239).

The numerical study of Gavini et al. (1983b), based on 169 strains, yielded five phena (A to E). Phenon A corresponded to *Erwinia carotovora*. Phenon B, which included strains of the *Enterobacter agglomerans* complex, was split into nine smaller phena (B1 to B9). Phenon B4 contained the type strains of *Erwinia herbicola* and *Erwinia milletiae*. Phenon B8 contained the type strain of *Erwinia ananas* and a reference strain of *Erwinia uredovora*. Phenon C corresponded to *Rahnella aquatilis*. Phenon D was split into three smaller phena (D1 to D3), with phenon D2 corresponding to *Enterobacter sakazakii*. Phenon E was split into five smaller phena (E1 to E5), with phenon E5 containing strains previously identified as *Escherichia adecarboxylata* (now *Leclercia adecarboxylata*).

A larger numerical study (Verdonck et al., 1987), based on 529 strains and including many type and reference strains, distributed 66 strains of the *Enterobacter agglomerans* complex into 21 phena. The correspondence between phena from both studies

1. Maintenance medium (g/liter): Bacto-peptone (Difco), 10.0, NaCl, 5.0: Bacto-agar (Difco), 10.0; pH 7.4. The medium should be dispensed into small (9.5–10 × 90 mm) screw-capped tubes.

(Gavini et al., 1983b; Verdonck et al., 1987) is given in Table BXII.γ.239.

The DNA relatedness work of Brenner et al. (1984a) revealed the extreme genomic diversity of the *Enterobacter agglomerans* complex: of 124 strains studied, 90 fell into 13 DNA groups (I to XIII) and 34 strains did not fit into any group. Furthermore, four groups (V, XI, XII, and XIII) were heterogeneous with respect to $\Delta(T_m)$ values. An interesting finding was that aerogenic strains and anaerogenic strains were not found in the same hybridization group.

Lind and Ursing (1986) identified 52 of 86 clinical isolates with *Enterobacter agglomerans sensu strictu* by DNA hybridization. In the same study, they demonstrated the synonymy of *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae*. This synonymy was confirmed by Beji et al. (1988) who, in addition, identified DNA group XIII (Brenner et al., 1984a) with *Enterobacter agglomerans sensu strictu*.

To separate *Enterobacter agglomerans/Erwinia herbicola* from the genera *Enterobacter* and *Erwinia*, a new genus, *Pantoea*, was proposed with *P. agglomerans* as the type species (Gavini et al., 1989b). The epithet *agglomerans* was retained because it was believed to have priority over epithet *herbicola*. Strains of DNA group III (Brenner et al., 1984a) or phenon 8 (Verdonck et al., 1987) were proposed as a new species, *Pantoea dispersa* (Gavini et al., 1989b). The DNA groups closest to *Pantoea agglomerans* are DNA groups II, III, IV, V, and VI (Lind and Ursing, 1986). It is interesting that strains of group II to VI and XIII are characterized by the presence of a glucose oxidation pathway that produces 2-ketogluconate from glucose (Bouvet et al., 1989). This finding has been extended to DNA group I (P.A.D. Grimont, unpublished observations). Thus, the genus *Pantoea* can be envisioned to include DNA groups I, II, IV, V, and VI in addition to groups XIII (*P. agglomerans*) and III (*P. dispersa*).

Erwinia ananas (Serrano 1928) corresponds to group VI of Brenner et al. (1984a) and was transferred to the genus *Pantoea* as *P. ananas* (Mergaert et al., 1993). The name was corrected to *P. ananatis* by Trüper and De' Clari, 1997). *Erwinia uredovora* belongs to that species.

Erwinia stewartii was included in the genus *Pantoea* as *P. stewartii* subsp. *stewartii* (Smith 1898) Mergaert et al. 1993. *P. stewartii* subsp. *indologenes* was a new subspecies composed of strains phenotypically different from *Erwinia stewartii* (and *P. stewartii* subsp. *stewartii*) and resembling *P. ananatis*. However, DNA hybridization and sequence studies indicate that both subspecies should belong to the same species.

The three species (*Pantoea punctata*, *P. citrea*, and *P. terrea*) described by Kageyama et al. (1992) have the ability to oxidize 2-ketogluconate to 2,5-diketogluconate. These species differ from the other *Pantoea* species in several biochemical or nutritional characteristics. Their phylogenetic position is as a branch on the border of the *Pantoea* cluster.

DNA group XI (Brenner et al., 1984a) was identified with *Escherichia adecarboxylata* by DNA relatedness (Izard et al., 1985). *Escherichia adecarboxylata* was transferred to a new genus *Leclercia*, as *L. adecarboxylata* (Tamura et al., 1986). DNA group VII (Brenner et al., 1984a) was close to (but distinct from) *Escherichia adecarboxylata* (*Leclercia adecarboxylata*) (Izard et al., 1985). In our laboratory, strains of DNA group VII were indistinguishable from strains of phenon E4 (Gavini et al., 1983b) by carbon source utilization tests (P.A.D. Grimont, unpublished observations). Thus, DNA group VII is a good candidate as a new species of *Leclercia*.

DNA group VIII (Brenner et al., 1984a) was 64% related to *Enterobacter persicina* (a close relative of *Erwinia rhapontici*).

DNA group IX (Brenner et al., 1984a) is identical with *Enterobacter cowanii* based on nutritional tests and *rpoB* sequence analysis (unpublished data).

More work is needed to characterize unclassified strains of the *E. agglomerans* complex.

Differentiation of the species of the genus *Pantoea*

The characteristics of named or unnamed species of *Pantoea* are given in Table BXII.γ.240 . The results of carbon source utilization tests given in Table BXII.γ.240 are mostly unpublished observations (P.A.D. Grimont and E. Ageron).

The genus *Pantoea* can be divided into two groups of species: (1) the *Pantoea* core group with *P. agglomerans*, *P. dispersa*, *P. ananatis* , and *P. stewartii* and (2) the "Japanese" group with *P. citrea*, *P. punctata*, and *P. terrea*. Species of the core group utilize the following substrates as sole carbon sources which the "Japanese" group cannot utilize: D-alanine, L-alanine, *myo*-inositol, DL-lactate, 1-*O*-methyl-β-D-glucoside, and L-serine. Furthermore, all species of the core group utilize L-arabinose and D-mannitol, whereas two species of the "Japanese" group fail to utilize these substrates. Species of the core group are negative for arginine dihydrolase and 2-ketogluconate dehydrogenase, whereas two species of the "Japanese" group are positive for arginine dihydrolase and all three species are positive for 2-ketogluconate dehydrogenase.

List of species of the genus Pantoea

1. **Pantoea agglomerans** (Ewing and Fife 1972) Gavini Mergaert, Beji, Mielcarek, Izard, Kersters and De Ley 1989b, 343^{VP} (*Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972, 10.)
   ag.glo' mer.ans. L. v. *agglomerare* to form into a ball; L. part. adj. *agglomerans* forming into a ball (referring to the occurrence of symplasmata bacteria in aggregates surrounded by a translucent sheath in anaerogenic strains).

   The species has all the characteristics given for the genus. Cells may form symplasmata. Strains grow well on nutrient agar at 30°C but not at 44°C. Some strains grow slowly at 4 or 41°C. The biochemical and nutritional characteristics are shown in Table BXII.γ.240. Key characteristics are: alkaline reaction in malonate broth; utilization of D-glucuronate and D-tartrate as sole carbon sources; inability to utilize *meso*-erythritol, gentiobiose, 5-ketogluconate, D-me-

TABLE BXII.γ.240. Characteristics of the named and unnamed species of the genus *Pantoea*.[a]

Characteristic	*Pantoea agglomerans*	*Pantoea ananatis*	*Pantoea citrea*	*Pantoea dispersa*	*Pantoea punctata*	*Pantoea stewartii* subsp. *stewartii*	*Pantoea stewartii* subsp. *indologenes*	*Pantoea terrea*	DNA group I	DNA group II	DNA group IV	DNA group V
Motility (36°C)	+	+	−	+	−	−	d	+	ND	d	d	d
Yellow pigment	d	+	−	d	−	+	+	−	ND	d	d	d
Indole production	−	+	−	−	−	−	+	−	−	−	−	−
Malonate (Leifson)	+	−	ND	−	ND	−	−	ND	−	d	−	d
β-Xylosidase test	d	−	ND	−	ND	ND	ND	ND	+	−	−	+
Voges–Prokauer	+	+	+	d	+	d	d	+	ND	d	d	d
Gelatin hydrolysis at 22°C	(+)	+	−	(d)	−	−	−	−	ND	d	d	+
Arginine dihydrolase	−	−	+	−	+	−	−	−	−	−	−	−
Phenylalanine deaminase	d	−	−	−	−	−	−	−	ND	+	d	d
Glucose dehydrogenase	+	+	+	+	+	ND	ND	+	+	+	+	+
Gluconate dehydrogenase	+	+	+	+	+	ND	ND	+	+	+	+	+
2-ketogluconate dehydrogenase	−	−	+	−	+	ND	ND	+	ND	−	−	−
Esculin hydrolysis	+	d	−	d	d	−	+	+	+	d	d	d
Nitrate reduced	+	d	+	d	+	−	−	+	ND	+	d	d
ONPG hydrolyzed	+	+	+	+	−	+	+	−	ND	ND	ND	ND
Acid from:												
L-Arabinose	+	+	ND	d	ND	+	+	ND	ND	+	+	+
D-Arabitol	+	+	d	+	−	−	+	−	ND	ND	ND	ND
Cellobiose	d	+	−	d	−	−	+	−	ND	d	d	d
Dulcitol	−	−	−	−	−	−	−	−	ND	−	−	d
meso-Erythritol	−	−	d	−	−	−	−	−	ND	ND	ND	ND
Glycerol	(d)	+	ND	(d)	ND	−	d	ND	ND	d	−	d
myo-Inositol	(d)	+	ND	d	ND	−	+	ND	ND	d	d	d
Lactose	d	+	+	d	−	−	+	−	+	d	d	d
Maltose	+	+	ND	+	ND	−	+	ND	ND	ND	ND	ND
D-Mannitol	+	+	+	+	−	+	+	−	ND	+	+	+
Melibiose	−	d	+	d	+	+	+	+	ND	ND	ND	ND
Raffinose	(d)	+	ND	−	ND	+	+	ND	ND	−	−	d
L-Rhamnose	+	d	−	+	−	−	d	−	ND	+	d	d
Salicin	+	+	d	(d)	−	−	+	+	ND	d	d	d
D-Sorbitol	−	+	d	−	−	−	−	d	ND	−	−	d
Sucrose	+	+	ND	+	ND	+	+	ND	ND	d	+	d
Trehalose	+	+	ND	+	ND	+	+	ND	ND	ND	ND	ND
D-Xylose	+	+	+	+	−	+	+	+	ND	+	d	+
Utilization of:												
trans-Aconitate	+	+	+	+	−	−	+	−	+	d	+	+
Adonitol	−	−	−	−	−	−	−	−	−	−	+	−
L-Arabinose	+	+	+	+	−	+	+	−	+	+	+	+
D-Arabitol	+	+	−	+	−	−	+	−	−	d	+	d
L-Arabitol	−	−	−	−	−	−	−	−	−	−	+	-
Betaine	−	−	−	−	−	−	+	−	−	−	−	−
Cellobiose	(d)	+	−	+	(+)	d	+	−	+	d	+	+
Citrate	d	+	+	+	(+)	−	+	+	+	d	+	+
Dulcitol	−	−	−	(d)	−	−	−	−	−	(d)	−	−
meso-Erythritol	−	−	+	+	−	−	−	−	−	−	d	−
L-Fucose	−	−	−	−	−	−	−	−	−	d	−	d
D-Galacturonate	(+)	+	−	+	−	d	+	−	+	+	d	+
Gentiobiose	−	+	−	+	+	−	d	−	+	d	d	−
D-Glucuronate	(+)	+	−	+	−	−	+	−	+	+	+	+
myo-Inositol	+	+	−	+	−	+	+	−	+	d	+	+
5-Ketogluconate	−	(+)	+	+	(+)	−	+	+	+	d	+	(d)
Lactose	−	+	(+)	−	−	d	+	−	+	d	−	(d)
Lactulose	−	+	+	−	−	d	+	−	+	d	−	d
D-Malate	(+)	d	+	(d)	−	+	+	−	(+)	(+)	−	(+)
Maltose	(+)	+	+	+	−	−	+	−	+	d	+	+
Maltotriose	(+)	+	+	+	−	−	+	−	+	d	+	+
D-Melibiose	−	+	−	−	−	+	+	−	−	d	−	−
1-*O*-Methyl-α-galactoside	−	(+)	−	−	−	+	+	−	−	d	−	−
3-*O*-Methyl-D-glucose	−	(d)	−	−	−	−	+	−	−	−	−	−

(*continued*)

TABLE BXII.γ.240. *(cont.)*

Characteristic	*Pantoea agglomerans*	*Pantoea ananatis*	*Pantoea citrea*	*Pantoea dispersa*	*Pantoea punctata*	*Pantoea stewartii* subsp. *stewartii*	*Pantoea stewartii* subsp. *indologenes*	*Pantoea terrea*	DNA group I	DNA group II	DNA group IV	DNA group V
1-*O*-Methyl-β-D-glucoside	(+)	+	−	+	−	+	+	−	+	+	+	+
Protocatechuate	−	+	−	−	−	−	+	−	−	−	−	−
Quinate	−	(+)	−	−	−	−	+	−	−	−	−	−
D-Raffinose	−	+	−	−	−	+	+	−	−	d	−	d
L-Rhamnose	+	d	−	+	−	d	d	−	+	+	d	+
D-Saccharate	(+)	(+)	−	+	−	−	+	−	+	+	+	+
D-Sorbitol	−	d	−	−	−	−	−	−	+	(d)	−	−
Sucrose	(+)	+	−	+	(+)	(+)	+	−	−	d	+	+
D-Tagatose	−	−	+	−	−	−	−	−	−	−	−	−
D-Tartrate	(+)	−	−	−	−	−	−	−	−	−	−	−
L-Tartrate	−	−	−	d	−	−	−	−	−	d	−	d
meso-Tartrate	(+)	d	−	d	−	+	+	−	−	d	−	(+)
Trigonelline	−	−	−	−	(d)	−	−	−	−	d	+	−
Xylitol	−	−	−	−	−	−	−	−	−	d	+	−
D-Xylose	+	+	−	+	−	+	+	−	+	+	−	+

[a]Symbols: +, 90–100% of strains positive in 1–2 days; (+), 90–100% of strains positive in 1–4 days; −, 90–100% of strains negative in 4 days; d, positive in 1–4 days; (d), positive in 3–4 days; ND, no data.

libiose and D-raffinose as sole carbon sources; and no production of indole and 2-ketogluconate dehydrogenase.

Isolated from plants, flowers, seeds, vegetables, water, soil and foodstuffs. Some strains are of human (wounds, blood, urine, internal organs) and animal origin. Some strains (synonym, *Erwinia milletiae*) have been reported to cause galls on *Wisteria floribunda* and *Wisteria japonica*; some strains cause galls on *Gypsophyla paniculata*, and some strains cause stalk and leaf necrosis on onion plants.

The mol% G + C of the DNA is: 55.1–56.8 (T_m).

Type strain: ATCC 27155, CCUG 539, CFBP 3845, CIP 57.51, DSM 3493, ICMP 12534, JCM 1236, LMG 1286, NCTC 9381.

GenBank accession number (16S rRNA): AB004691, AJ233423.

2. **Pantoea ananatis** corrig. (Serrano 1928) Mergaert, Verdonck and Kersters 1993, 170[VP] (*"Bacillus ananas"* Serrano 1928, 271; *Erwinia ananas* (sic) Serrano 1928; *Pantoea ananas* (sic) Mergaert, Verdonck and Kersters 1993, 170.)
a' na.na.tis. M.L. n. *ananas* generic name of the pineapple.

The species has all the characteristics given for the genus. The biochemical and nutritional characteristics are given in Table BXII.γ.240. Key characteristics are: indole production; no alkaline reaction in malonate broth; utilization of gentiobiose, D-glucuronate, 5-ketogluconate, lactose, lactulose, D-melibiose, 1-*O*-methyl-α-galactoside, protocatechuate, quinate and D-raffinose as sole carbon sources; utilization of D-sorbitol by most strains; and no utilization of *meso*-erythritol and D-tartrate.

Strains formerly classified as *Erwinia uredovora* show a stronger proteinase activity and produce a DNase.

The mol% G + C of the DNA is: 53.6–56.4 (T_m).

Type strain: ATCC 33244, CFBP 3612, CIP 105207, LMG 2665, NCPPB 1846.

GenBank accession number (16S rRNA): U80196.

3. **Pantoea citrea** Kageyama, Nakae, Yagi and Sonoyama 1992, 209[VP]
ci' tre.a. M.L. adj. *citrea* of citrus.

The cell morphology and colonial morphology are as given for the genus. The cells are nonmotile. Either nicotinic acid or nicotinamide is required for growth. Good growth occurs at 20–34°C; no growth occurs at 41°C. Colonies grown on nutrient agar at 30°C for 2 d are pale beige to pale reddish yellow. Physiological and nutritional characteristics are presented in Table BXII.γ.240. Key characteristics are: production of 2-ketogluconate dehydrogenase; positive arginine dihydrolase reaction; hydrolysis of ONPG but not esculin; utilization of L-arabinose, *meso*-erythritol, maltose, and D-tagatose as sole carbon and energy sources; and no utilization of gentiobiose. Isolated from mandarin oranges.

The mol% G + C of the DNA is: 49.7 (HPLC).

Type strain: SHS 2003, ATCC 31623, CIP 105599, CCUG 30156, DSM 13699.

4. **Pantoea dispersa** Gavini, Mergaert, Beji, Mielcarek, Izard, Kersters and De Ley 1989b, 344[VP]
dis.per' sa. L. v. *dispergere* to spread, to scatter; L. fem. part. adj. *dispersa* spread, scattered.

The species has all the characteristics of the genus. Strains grow well on nutrient agar at 30 and 41°C but not at 44°C or 4°C. Key characteristics are: no alkaline reaction in malonate broth; utilization of *meso*-erythritol, gentiobiose, D-glucuronate and 5-ketogluconate as sole carbon sources; no utilization of D-melibiose, D-raffinose, and D-tartrate as sole carbon sources; and no production of indole and 2-ketogluconate dehydrogenase. Isolated from plant surfaces, seeds, humans, and the environment.

The mol% G + C of the DNA is: 56.5–60.6 (T_m).

Type strain: ATCC 14589, CCUG 25232, CIP 103338, DSM 30073, LMG 2603.

5. **Pantoea punctata** Kageyama, Nakae, Yagi and Sonoyama 1992, 209^{VP}

punc.ta' ta. L. n. *punctum* a point; M.L. adj. *punctata* full of points.

The cell morphology and colonial morphology are as given for the genus. Cells are nonmotile. Either nicotinic acid or nicotinamide is required for growth. Good growth occurs at 20–34°C; no growth occurs at 41°C. Colonies grown on nutrient agar at 30°C for 2 d are pale beige to pale reddish yellow. Yellow pigment is not produced on nutrient agar. Physiological and nutritional characteristics are presented in Table BXII.γ.240. Key characteristics are: production of 2-ketogluconate dehydrogenase; positive arginine dihydrolase reaction; hydrolysis of esculin by some strains; no hydrolysis of ONPG; utilization of gentiobiose as a sole source of carbon and energy; and no utilization of L-arabinose, *meso*-erythritol, maltose, and D-tagatose. Isolated from mandarin oranges.

The mol% G + C of the DNA is: 50.0–50.3 (HPLC).

Type strain: SHS 2006, ATCC 31626, CIP 105598, DSM 13700.

6. **Pantoea stewartii** (Smith 1898) Mergaert, Verdonck and Kersters 1993, 170^{VP} (*"Pseudomonas stewartii"* Smith 1898, 422; *Erwinia stewartii* (Smith 1898) Dye 1963b, 504.)

stew.art' i.i. M.L. en. n. *stewartii* of Stewart; named after F.C. Stewart.

The species has all the characteristics given for the genus, except that some strains produce a capsular polysaccharide. The species is divided into two subspecies that differ strongly in vigor. Previous information about the characteristics of *Erwinia stewartii* applies only to *P. stewartii* subsp. *stewartii*, not to *P. stewartii* subsp. *indologenes*, which can hardly be differentiated from *P. ananatis* by biochemical and nutritional properties. Common characteristics of the species are listed in Table BXII.γ.240. Key characteristics are: production of a yellow pigment; no alkaline reaction in malonate broth; no reduction of nitrate to nitrite; acid production from L-arabinose, sucrose, and D-mannitol but not from D-sorbitol; utilization of L-alanine, L-arabinose, *myo*-inositol, DL-lactate, D-mannitol, D-melibiose, 1-*O*-methyl-α-galactoside, 1-*O*-methyl-β-galactoside, 1-*O*-methyl-β-D-glucoside, D-raffinose, L-serine, sucrose, *meso*-tartrate, and D-xylose as sole sources of carbon and energy; and no utilization of *meso*-erythritol and D-sorbitol. Isolated from *Zea mays*, several other grasses, *Ananas comosus*, and beetles.

The mol% G + C of the DNA is: 53.6–56.4 (T_m).

Type strain: ATCC 8199, CIP 104005, DSM 30176, LMG 2715, NCPPB 2295.

a. **Pantoea stewartii** *subsp.* **stewartii** (Smith 1898) Mergaert, Verdonck and Kersters 1993, 170^{VP} (*"Pseudomonas stewartii"* Smith 1898, 422; *Erwinia stewartii* (Smith 1898) Dye 1963b, 504.)

The biochemical and nutritional characteristics are given in Table BXII.γ.240. A few strains grow at 4°C. Some strains grow at 37°C, but no strains grow at 41°C. Key characteristics are: lack of motility; no production of indole; no hydrolysis of esculin; no acid production from D-arabitol, cellobiose, *myo*-inositol, lactose, maltose, L-rhamnose and salicin; and no utilization of D-arabitol, betaine, citrate, D-glucuronate, 5-ketogluconate, maltose, 3-*O*-methyl-D-glucose, protocatechuate, quinate, and D-saccharate as sole carbon and energy sources.

Causative agent of Stewart's bacterial wilt of corn, a vascular disease of *Zea mays*. Also isolated from the insect vector, the corn flea beetle *Chaetocnema pulicaria*.

The mol% G + C of the DNA is: 54.6–55.1 (T_m).

Type strain: ATCC 8199, CIP 104005, DSM 30176, LMG 2715, NCPPB 2295.

GenBank accession number (16S rRNA): Z96080.

b. **Pantoea stewartii** *subsp.* **indologenes** Mergaert, Verdonck and Kersters 1993, 171^{VP}

in.do.lo' gen.es. M.L. n. *indolum* indole; Gr. n. *gennao* to produce; M.L. adj. *indologenes* indole producing.

The biochemical and nutritional characteristics are given in Table BXII.γ.240. Many strains grow at 4 and 37°C and some strains grow at 41°C, but few strains grow at 44°C. Key characteristics are: motility; production of indole; hydrolysis of esculin; acid production from D-arabitol, cellobiose, *myo*-inositol, lactose, maltose, D-rhamnose and salicin; and utilization of L-arabitol, betaine, citrate, D-glucuronate, 5-ketogluconate, maltose, 3-*O*-methyl-D-glucose, protocatechuate, quinate and D-saccharate as sole carbon and energy sources.

Thought to cause leaf spot on foxtail millet (*Setaria italica*) and pearl millet (*Pennisetum americanum*) and rot of *Ananas comosus*. Also isolated from cluster bean (*Cyamopsis tetragonolobus*).

The mol% G + C of the DNA is: 56.4 (T_m).

Type strain: ATCC 51785, CIP 104006, LMG 2632.

GenBank accession number (16S rRNA): Y13251.

7. **Pantoea terrea** Kageyama, Nakae, Yagi and Sonoyama 1992, 210^{VP}

ter' re.a. L. n. *terra* soil; L. adj. *terrea* of soil.

The cell morphology and colonial morphology are as given for the genus. The cells are motile by means of one or two lateral flagella. Colonies grown on nutrient agar at 30°C for 2 d are pale beige to pale reddish yellow. Yellow pigment is not produced on nutrient agar. Physiological and nutritional characteristics are presented in Table BXII.γ.240. Key characteristics are: production of 2-ketogluconate dehydrogenase; negative arginine dihydrolase reaction; hydrolysis of esculin but not ONPG; and no utilization of L-arabinose, *meso*-erythritol, gentiobiose, maltose, and D-tagatose as sole carbon and energy sources. Isolated from soil in Japan

The mol% G + C of the DNA is: 51.0–51.9 (HPLC).

Type strain: SHS 2008, ATCC 31628, CCUG 30161, CIP 105600, DSM 13701.

Genus XXIV. **Pectobacterium** *Waldee 1945, 469[AL] emend. Hauben, Moore, Vauterin, Steenakcers, Mergaert, Verdonck and Swings 1999a, 1*

LYSIANE HAUBEN, FREDERIQUE VAN GIJSEGEM AND JEAN SWINGS

Pec.to.bac.te' ri.um. Gr. dim. neut. n. *bakterion* a small rod; M.L. neut. n. *Pectobacterium* a pectolytic bacterium.

Straight rods, 0.5–1.0 × 1.0–3.0 μm, rounded ends; occur singly or in pairs. Gram-negative. Motile by peritrichous flagella. Fermentative. Facultatively anaerobic. Optimum growth temperature, 27–30°C; maximum temperature for growth is 40°C. Oxidase negative. Catalase positive. Acid is produced from ***N*-acetylglucosamine**, fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, D-ribose, salicin, and sucrose but not from adonitol, L-arabitol, D-lyxose, α-methylmannoside, L-sorbose, starch, or D-tagatose. Utilize acetate, arbutin, fructose, fumarate, D-galactose, D-glucose, glycerol, malate, D-mannitol, mannose, β-methylglucoside, ribose, salicin, succinate, and sucrose but not adipate, benzoate, betaine, butanol, gallate, methanol, oxalate, propionate, or sorbose as carbon and energy-yielding sources. Utilize L-alanine, allantoin, γ-aminobutanic acid, ammonium chloride, arginine, asparagine, asparaginic acid, citrulline, glucosamine, glutamine, L-glutaminic acid, glutathione, glycine, glycylglycine, histidine, leucine, L-methionine, phenylalanine, L-serine, L-tryptophan, tyrosine, and urea, but not anthranilic acid, betaine, choline, cysteamine, hydroxyproline, kynureninic acid, quinolinic acid, sarcosine, spermidine, spermine, trigonelline, or trimethylammonium as nitrogen sources. No decarboxylases are formed for arginine, lysine or ornithine. Do not possess tryptophan deaminase or urease. **Hydrolyze esculin but not starch.**

The species of the genus *Pectobacterium* comprise a distinct phylogenetic group, as determined by 16S rRNA gene sequence comparisons, and are characterized by 17 signature nucleotides (Table BXII.γ.223 in Genus *Erwinia*).

Pectobacterium species cause plant diseases that include blights, cankers, die back, leaf spots, wilts, discoloration of plant tissues, and especially soft rots variously described as stalk rot, crown rot, stem rot, or fruit collapse. Ingress by the pathogen generally occurs through natural openings and wounds. Soft rot is characterized by the breakdown of the plant cell wall, leading to maceration of the parenchyma, loss of electrolytes, and cell death. Pectinases and pectate lyases are mainly responsible for these symptoms.

The mol% G + C of the DNA is: 50.5–56.1.

Type species: **Pectobacterium carotovorum** (Jones 1901) Waldee 1945, 469 emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Erwinia carotovora* (Jones 1901) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171; *Bacillus carotovorus* Jones 1901, 12.)

FURTHER DESCRIPTIVE INFORMATION

Metabolic features are the same as for the genus *Erwinia*. Decarboxylases for arginine, lysine, or ornithine are not present except in a few (usually 5% or less) strains of *Pectobacterium carotovorum* and *Pectobacterium chrysanthemi*.

Pectate lyases are produced by all species except by *Pectobacterium cypripedii*. Cellulases (Cx) are produced by strains of *P. carotovorum*, *P. carotovorum* subsp. *atrosepticum*, and *P. chrysanthemi* (El-Helaly et al., 1979). *P. carotovorum* and *P. chrysanthemi* produce pectinases, cellulases, hemicellulases, and proteases (Garibaldi and Bateman, 1973; Bertheau et al., 1984; Collmer and Keen, 1986; Ried and Collmer, 1986; Wandersman et al., 1986; Willis et al., 1987). *P. carotovorum* produces an endopolygalacturonase (Lei et al., 1985; Willis et al., 1987) as well. The genes encoding these enzymes that have been mapped are located on the chromosome (Chatterjee et al., 1981; Hugouvieux-Cotte-Pattat et al., 1996). In most *Pectobacterium* species, production of the plant cell wall degrading enzyme pectin lyase (Pnl) is activated by DNA-damaging agents such as mitomycin C (MC), nalidixic acid, and UV light (Liu et al., 1994b).

Naturally occurring plasmids have been detected in strains of *P. carotovorum* and *P. chrysanthemi*, and plasmids from bacteria other than *Pectobacterium* have been introduced into strains of the foregoing *Pectobacterium* species (Lacy and Leary, 1979; Chatterjee and Starr, 1980). Plasmid-mediated transfer of chromosomal genes by conjugation also has been reported for strains of *P. carotovorum* and *P. chrysanthemi*.

Plasmid pULB113 (Van Gijsegem and Toussaint, 1982) mediates chromosomal mobilization and R-prime formation in *P. carotovorum* and *P. chrysanthemi* (Chatterjee et al., 1985).

Virulent or temperate phages have reported to be active against strains of *P. carotovorum* (Chapman et al., 1951; Faltus and Kishko, 1980; Pirhonen and Palva, 1988; Gross et al., 1991; Toth et al., 1993) and *P. chrysanthemi* (Paulin and Nassan, 1978). Bacteriocinogeny or production of bacteriocin-like substances has been noted for strains of *P. carotovorum* (Itoh et al., 1978), *P. chrysanthemi* (Echandi and Moyer, 1979), and *Pectobacterium* species from sugar beet (Stanghellini et al., 1977). Bacteriocin-resistant mutants of *P. chrysanthemi* have been isolated (Expert and Toussaint, 1985).

Lipopolysaccharide defective mutants of *P. carotovorum* (Pirhonen and Palva, 1988) and genetically engineered kanamycin resistant strains of *P. carotovorum* (Scanferlato et al., 1989; Orvos et al., 1990) have been isolated.

Antisera prepared against live or heat-killed cells, nonpurified or purified immunogens have been used for the differentiation or identification of all *Pectobacterium* species except *P. cypripedii* (Schaad, 1979). Serogroups have been determined for *P. carotovorum* (De Boer et al., 1979) and *P. chrysanthemi* (Samson and Nassan-Agha, 1978; Yakrus and Schaad, 1979).

More recently, a conductimetric assay was developed for automated detection of *P. carotovorum* subsp. *atrosepticum*, *P. carotovorum* subsp. *carotovorum*, and *P. chrysanthemi* (Fraaije et al., 1996a, 1997), and a luminescence-based assay is available for the detection of *P. carotovorum* (Grant et al., 1992; McLennan et al., 1992).

A strain of *P. carotovorum* subsp. *carotovorum* was found to produce the antibiotic 1-carbapen-2-em-3-carboxylic acid (Bainton et al., 1992b).

16S rDNA sequence analyses of the species of the genus *Pectobacterium* by Kwon et al. (1997) and Hauben et al. (1998a) agree very well, except for the type strain of *P. cypripedii*, and situate the genus *Pectobacterium* within the *Enterobacteriaceae*, closely related to the genera *Pantoea*, *Erwinia*, *Brenneria*, and *Enterobacter* (Fig. BXII.γ.201 and Table BXII.γ.223 of the genus *Erwinia*). The sequence of the type strain of *P. cypripedii*, sequenced by Kwon et al. (1997) (ATCC 29267) and Hauben et al. (1998a) (LMG 2657), differ in 53 nucleotides. The sequence reported by Hauben et al. (1998a) places *P. cypripedii* in the *Pectobacterium* cluster.

Enrichment and Isolation Procedures

The isolation procedure is the same as for the genus *Erwinia*.

The isolation of some *Pectobacterium* species can be facilitated by use of selective-differential media, but such media are usually not necessary. Selective media have been developed for the isolation of pectolytic bacteria (Kelman and Dickey, 1980). The crystal violet pectate (CVP)[1] medium is commonly used. Miller-Schroth (Miller and Schroth, 1972) medium modified by replacement of most of the agar by sodium polypectate selectively allows the growth of pectolytic species. NaOH (105%) and MOPS (3-(-*N*-morpholino)propanesulfonic acid; 0.4%) are added to raise the pH and buffer the medium (Pierce and McCain, 1992). *P. chrysanthemi* strains isolated from plants grow well on LB medium[2].

Maintenance Procedures

Stock cultures of *Pectobacterium* species should be grown on standard media of choice at 25–30°C. The cultures can be maintained for short-term storage in a refrigerator (4–5°C); some strains of *P. chrysanthemi* are nonviable after 3–4 weeks at 4°C, but remain viable for longer periods when stored at 12°C. Long-term preservation is the same as for the genus *Erwinia*.

Differentiation of the genus *Pectobacterium* from other genera

Characteristics that differentiate *Pectobacterium* from the genera *Erwinia*, *Pantoea*, and *Brenneria* are given in Table BXII.γ.223 of the genus *Erwinia*. Apart from their pectolytic nature, it is very difficult to differentiate them phenotypically; genomic methods are recommended for differentiation.

Taxonomic Comments

The species of the genus *Pectobacterium* were formerly classified under *Erwinia*. We refer the reader to the taxonomic comments section of the genus *Erwinia* for a discussion on this issue.

Strains of *P. chrysanthemi* have been isolated from numerous plant species and cultivars (Dickey, 1981). Six pathovars (pathovar *chrysanthemi*, pathovar *dianthicola*, pathovar *dieffenbachiae*, pathovar *paradisiaca*, pathovar *parthenii*, and pathovar *zeae*) have been designated for *P. chrysanthemi* (Dye et al., 1980). The relationship between pathogenicity, phenotypic properties and serological reactions of strains of the pathovars is not clear (Samson and Nassan-Agha, 1978; Yakrus and Schaad, 1979; Dickey, 1981; Thomson et al., 1981).

Differentiation of the species of the genus *Pectobacterium*

The differential characteristics of the species of *Pectobacterium* are given in Tables BXII.γ.241, BXII.γ.242, BXII.γ.243, and BXII.γ.244. Only a small number (eight) of strains of *P. cypripedii* have been studied.

List of species of the genus Pectobacterium

1. **Pectobacterium carotovorum** (Jones 1901) Waldee 1945, 469[AL] emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Erwinia carotovora* (Jones 1901) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171; *Bacillus carotovorus* Jones 1901, 12.)
ca.ro.to′vo.rum. L. n. *carota* carrot; L. v. *voro* to devour; M.L. adj. *carotovorum* carrot-devouring.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.241, BXII.γ.242, BXII.γ.243, and BXII.γ.244. Causes rotting, particularly of storage tissues, of a wide variety of plants and causes a vascular and parenchymal disease (blackleg) of potato plants. The small diffusible autoinducer signal molecule *N*-(β-ketocaproyl) homoserine lactone (KHL) acts as a molecular control signal for the expression of genes controlling carbapenem antibiotic biosynthesis (Bainton et al., 1992a). The *pnl* gene, encoding pectin lyase (Ohnishi et al., 1991), and the *peh* gene, encoding polygalacturonase (Peh) (Lei et al., 1992) of *P. carotovorum*, have been located and characterized.

   Subspecies can be differentiated by (1) PCR with primers Y1 (5′-TTACCGGACGCCGAGCTGTGGCGT-3′) and Y2 (5′-CAGGAAGATGTCGTTATCGCGAGT-3′), annealing at 65°C, and (2) restriction fragment length polymorphism (RFLP) of a *pel* gene identifying *P. carotovorum* subsp. *atrosepticum* and *P. carotovorum* subsp. *wasabiae* strains (Darrasse et al., 1994b). The pectate lyase (*pel*) genes I and III were characterized by Nikaido et al. (1985) and Yoshida et al. (1991), respectively. RAPD-PCR can differentiate *P. carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *atrosepticum* (Maki-Valkama and Karjalainen, 1994; Parent et al., 1996) and a DNA probe, isolated from a genomic library, can differentiate the subspecies *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *atrosepticum*, and *P. carotovorum* subsp. *betavasculorum* (Ward and De Boer, 1990).

   The bacteriocin carotovoricin was identified (Itoh et al., 1982) and the gene *recA*, which is required for its induction (Zink et al., 1985, Zhao and McEntee, 1990). Monoclonal antibodies were developed for detection of *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *atrosepticum*, and *P. carotovorum* subsp. *betavasculorum* (Ward and De Boer, 1989; Murray et al., 1990a; Vernon-Shirley and Burns, 1992). *Drosophila melanogaster* Meigen and *Drosophila rucksii* Coquillett can function as vectors for *P. carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *atrosepticum* (Brewer et al., 1981).
   The mol% G + C of the DNA is: 50.5–53.1 (T_m, Bd).
   Type strain: ATCC 15713, NCPPB 312, ICMP 5702.

   a. **Pectobacterium carotovorum** *subsp.* **carotovorum** (Jones 1901) Waldee 1945, 469[AL] (*Erwinia carotovora* subsp. *carotovora* Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171.)

      Characteristics distinguishing this subspecies from the other *Pectobacterium carotovorum* subspecies are indicated in Table BXII.γ.243.

      The production of extracellular enzymes such as pectate lyase (Pel), polygalacturonase (Peh), cellulase (Cel), and protease (Prt) is activated by the cell density (quorum)-sensing signal, *N*-(3-oxohexanoyl)-L-homoserine lactone (HSL), and/or CarR (Barras et al., 1994). *RmsA*, a global repressor gene, controls the production of these

1. CVP medium consists of 1N NaOH, 4.5 ml; 10% $CaCl_2 \cdot H_2O$, 3 ml ; Bacto-agar, 1.5 g; $NaNO_3$, 1 g; Bacto-yeast extract, 0.05 g; blended with 300 ml boiling distilled water for 15 s, to which 15 g sodium polypectate and 200 ml boiling distilled water is added and blended (Cuppels and Kelman, 1974; Woodward and Robinson, 1990).

2. LB medium consists of (per l distilled water) tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g.

TABLE BXII.γ.241. Diagnostic characteristic of the *Pectobacterium* species[a]

Characteristic	1. *P. carotovorum*	2. *P. cacticida*	3. *P. chrysanthemi*	4. *P. cypripedii*
Pectinase	+	+	+	−
Reducing sugars produced from sucrose	v	−	+	nd
Indole	−	−	+	−
Growth at 37°C	v	+	+	nd
Growth at 40°C	−	+	nd	nd
Production of acetoin	v	+	+	nd
Arginine dihydrolase	−	nd	d	nd
Caseinase	v	nd	+	−
Gelatinase	v	−	+	nd
Lecithinase	−	nd	+	−
Phenylalanine deaminase	−	nd	−	+
Phosphatase	−	d	+	nd
Acid production from:				
Lactose	+	nd	+	−
Malonate	−	nd	d	nd
Maltose	v	nd	−	+
Raffinose	v	nd	+	−
Trehalose	+	nd	−	+
Utilization of carbon sources:				
D-Arabinose	−	−	d	nd
L-Arabinose	+	−	+	nd
Cellobiose	+	−	+	nd
Gentiobiose	+	d	−	nd
α-Methylglucoside	d	nd	−	nd
myo-Inositol	+	−	nd	nd
Malonate	−	+	+	nd
Melibiose	v	−	+	nd
Raffinose	+	−	+	nd
Tartrate	−	−		+
Sensitivity toward:				
Erythromycin	−	−	+	+

[a]For symbols see standard definitions: nd, not determined.

enzymes and tissue macerating ability (Bainton et al., 1992b; Cui et al., 1995; McGowan et al., 1995; Mukherjee et al., 1996). The gene *aepA* (activator of extracellular protein production) controls the production of pectolytic enzymes Pel, Peh, Cel, and Prt (Liu et al., 1993c; Murata et al., 1994), and the *out* gene cluster encoding the proteins of the type II or general secretory pathway (GSP) apparatus is required for secretion of pectinases and cellulases (Reeves et al., 1993; Thomas et al., 1997). The *pnlA* gene, encodes DNA damage-inducible pectin lyase (Chatterjee et al., 1991) and *rdgB* encodes a transcriptional factor that specifically interacts with the pectin lyase structural gene *pnlA* promoter/regulatory region (Liu et al., 1997e). The *pnlA* gene is activated by DNA-damaging agents like RecA, RdgA, and RdgB. RdgA and RdgB also control bacteriocin production, phage release, and cell lysis (Barras et al., 1994).

A pleiotrophic reduced virulence (Rvi-negative) mutant was found to be defective in flagella assembly proteins (Mulholland et al., 1993). Several monoclonal antibodies have been produced against *P. carotovorum* subsp. *carotovorum* strains that can be used for identification and detection by Ouchterlony double diffusion (ODD), indirect immunofluorescence (IIF), and enzyme-linked immunosorbent assay (ELISA) (Alarcon et al., 1995), and for detection by reverse passive hemagglutination (RPH) (Koehm and Eggers-Schumacher, 1995).

Pseudomonas fluorescens strains are antagonistic (biological control agent) to *P. carotovorum* subsp. *carotovorum* (El Hendawy et al., 1998). Causes rotting, particularly of storage tissues, of a wide variety of plants, e.g., mushroom (*Agaricus* sp.), century plant (*Agave* sp.), mustard (*Brassica campestris*, *B. nigra*, and *B. juncea*), Chinese cabbage (*Brassica chinensis*), cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*Brassica oleracea* var. *capitata*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), broccoli (*Brassica oleracea* var. *italica*), turnip (*Brassica rapa* var. *rapa*), and ornamental plants. Larger fleshy organs are particularly susceptible and, once infected, they usually become softened to a pulp very quickly.

The mol% G + C of the DNA is: 50.5–53.1 (T_m, Bd).

Type strain: ATCC 15713, DSM 30168, LMG 2404.

GenBank accession number (16S rRNA): Z96089, U80197.

b. **Pectobacterium carotovorum** *subsp.* **atrosepticum** (van Hall 1902) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 393) (*Erwinia carotovora* subsp. *atroseptica* (van Hall 1902) Dye 1969a, 81; *Bacillus atrosepticus* van Hall 1902, 134.)

at.ro.sep' ti.cum. L. adj. *ater* black; Gr. adj. *septicus* producing a putrefaction; M.L. adj. *atrosepticum* producing a black rot.

Characteristics distinguishing this subspecies from the other *P. carotovorum* subspecies are indicated in Table BXII.γ.243. Several regions of the bacterial DNA of *P. carotovorum* subsp. *atrosepticum* seem to be subspecies-specific and can be used for identification by (1) genomic subtraction of DNA probes (Darrasse et al., 1994a);

TABLE BXII.γ.242. Additional characteristics of the *Pectobacterium* species[a]

Characteristic	1. *P. carotovorum*	2. *P. cacticida*	3. *P. chrysanthemi*	4. *P. cypripedii*
$NO_3^- \rightarrow NO_2^-$	+	+	+	nd
Production of aminopeptidase	nd	+	+	nd
Growth in 5%NaCl	v	nd	−	nd
Oxidase	−	−	−	−
Catalase	+	+	+	+
Citrate (Simmons)	v	d	+	nd
H_2S from cysteine	v	nd	v	−
β-Galactosidase	v	nd	+	nd
Arginine decarboxylase	−	−	−	−
Lysine decarboxylase	−	−	−	−
Ornithine decarboxylase	−	−	−	−
Esculin hydrolase	+	+	+	+
Starch hydrolase	−	−	−	−
Tryptophan deaminase	−	−	−	−
Urease	−	−	−	−
Acid production from:				
Adonitol	−	−	−	−
Amygdalin	nd	+	+	nd
D-Arabitol	v	nd	−	nd
L-Arabitol	−	−	−	−
L-Arabinose	nd	+	+	nd
Arbutin	nd	+	+	nd
Cellobiose	nd	+	+	nd
Citrate	v	nd	+	nd
Dulcitol	nd	−	−	−
Erythritol	nd	−	−	nd
Esculin	nd	+	+	nd
Fructose	+	+	+	+
DL-Fucose	nd	−	−	nd
Galactose	+	+	+	+
Gentiobiose	+	+	nd	nd
D-Glucose	+	+	+	+
N-acetylglucosamine	+	+	+	+
Glycerol	nd	+	+	+
Glycogen	nd	−	−	nd
5-Ketogluconate	−	−	nd	nd
Inulin	v	nd	d	−
D-Lyxose	−	−	−	−
D-Mannose	+	+	+	+
α-Methylmannoside	−	−	−	−
Melezitose	−	−	−	nd
Melibiose	nd	v	+	nd
α-Methylglucoside	nd	v	−	−
β-Methylxyloside	nd	−	−	nd
Palatinose	v	nd	−	nd
L-Rhamnose	+	+	+	+
D-Ribose	+	+	+	+
Salicin	+	+	+	+
Sorbitol	v	nd	−	nd
L-Sorbose	−	−	−	−
Starch	−	−	−	−
Sucrose	+	+	+	+
D-Tagatose	−	−	−	−
Xylitol	nd	−	−	nd
D-Xylose	nd	+	+	nd
L-Xylose	nd	−	−	nd
Utilization of carbon sources:				
Acetate	+	+	+	+
Adipate	−	−	−	−
Amygdalin	d	d	−	nd
L-Arabinose	nd	+	+	+
D-Arabitol	v	−	−	nd
Arbutin	+	+	+	+
Benzoate	−	−	−	−
Betaine	−	−	−	−
Butanol	−	−	−	−
Citrate	+	+	+	nd
Dulcitol	nd	−	−	−
Erythritol	nd	−	−	nd
Esculin	nd	+	+	nd
Fructose	+	+	+	+

(*continued*)

TABLE BXII.γ.242. *(cont.)*

Characteristic	1. *P. carotovorum*	2. *P. cacticida*	3. *P. chrysanthemi*	4. *P. cypripedii*
DL-Fucose	nd	−	−	nd
Fumarate	+	+	+	+
Galactose	+	+	+	+
Gallate	−	−	−	−
Gluconate	nd	+	+	+
Glucose	+	+	+	+
DL-Glycerate	nd	nd	nd	nd
Glycerol	d	+	+	+
Glycogen	−	nd	nd	nd
α-Ketoglutarate	nd	+	nd	nd
Inulin	v	−	d	nd
Lactose	d	+	d	nd
Lyxose	−	nd	nd	nd
Mallate	+	+	+	+
Maltose	d	d	−	nd
Mannitol	+	+	+	+
Mannose	+	+	+	+
Melezitose	−	nd	nd	nd
Methanol	−	−	−	−
β-Methylglucoside	+	+	+	+
Mucate	nd	nd	+	nd
Oxalate	−	−	−	−
Pectinic acid	+	+	+	nd
Propionate	−	−	−	−
L-Rhamnose	+	+	+	nd
Ribose	+	+	+	+
Saccharose	+	nd	nd	nd
Salicin	+	+	+	+
Sorbitol	v	−	−	nd
Sorbose	−	−	−	−
Starch	−	nd	nd	nd
Succinate	+	+	+	+
Sucrose	+	+	+	+
D-Tagatose	−	nd	nd	nd
meso-Tartrate	−	−	nd	nd
Trehalose	d	+	v	nd
Triacetine	ND	−	nd	nd
D-Turanose	v	−	−	nd
Xylitol	−	−	nd	nd
D-Xylose	d	ND	+	nd
Utilization of nitrogen sources:				
Adenine	nd	nd	+	nd
Alanine	+	+	+	+
Allantoin	+	+	+	+
Ammonium chloride	+	+	+	+
Anthranilic acid	−	−	−	−
Arginine	+	+	+	+
Asparagine	+	+	+	+
Asparginic acid	+	+	+	+
Betaine	−	−	−	−
γ-Aminobutanic acid	+	+	+	+
Carnosine	nd	nd	+	nd
Choline	−	−	−	−
Citrulline	+	+	+	+
Creatine	−	−	nd	nd
Cysteamine	−	−	−	−
Glucosamine	+	+	+	+
Glutamine	+	+	+	+
Glutaminic acid	+	+	+	+
Glutathion	+	+	+	+
Glycine	+	+	+	+
Glycylglycine	+	+	+	+
Guanine	−	−	nd	nd
Histidine	+	+	+	+
Hydroxypyroline	−	−	−	−
Kynureninic acid	−	−	−	−
Leucine	+	+	+	+
Methionine	+	+	+	+
Nicotinic acid	−	−	nd	nd
Octopine	nd	nd	+	nd
Ornithine	nd	nd	+	nd

(*continued*)

TABLE BXII.γ.242. *(cont.)*

Characteristic	1. *P. carotovorum*	2. *P. cacticida*	3. *P. chrysanthemi*	4. *P. cypripedii*
Phenylalanine	+	+	+	+
Quinolinic acid	−	−	−	−
Sarcosine	−	−	−	−
Serine	+	+	+	+
Spermidine	−	−	−	−
Spermine	−	−	−	−
Taurine	nd	nd	−	nd
Threonine	nd	nd	+	nd
Trigonelline	−	−	−	−
Trimethylammonium	−	−	−	−
Tryptamine	−	−	nd	nd
Tryptophane	+	+	+	+
Tyrosine	+	+	+	+
Ureum	+	+	+	+
Valine	nd	nd	+	nd
Xanthin	−	−	nd	nd
Sensitivity toward:				
Amoxycillin	+	+	nd	nd
Cephalotine	+	+	nd	nd

[a]For symbols see standard definitions; nd, not determined.

TABLE BXII.γ.243. Differentiation of *Pectobacterium carotovorum* subspecies[a]

Characteristic	*P. carotovorum* subsp. *carotovorum*	*P. carotovorum* subsp. *atrosepticum*	*P. carotovorum* subsp. *betavasculorum*	*P. carotovorum* subsp. *odoriferum*	*P. carotovorum* subsp. *wasabiae*
Reducing sugars produced from sucrose	−	+	+	+	−
Methyl red	+	+	−	nd	+
Growth in 5% NaCl	+	+	+	nd	−
Growth at 37°C	+	−	+	+	nd
Utilization of citrate	+	+	−	nd	+
H_2S from cysteine	v	v	v	−	+
Acetoin production	+	v	+	+	−
Citrate (Simmons)	+	+	−	+	
Caseinase	+	−	−	nd	+
β-Galactosidase	+	+	+	+	−
Gelatinase	+	−	v	+	+
Acid production from:					
d-Arabitol	−	−	−	+	nd
Citrate	+	+	−	nd	+
α-Methylglucoside	−	+	v	+	−
Gluconate	−	+	−	nd	nd
myo-Inositol	−	−	+	−	−
Inulin	−	−	+	−	−
Maltose	−	+	+	nd	−
Melibiose	+	+	−	+	−
Palatinose	−	+	+	+	nd
Raffinose	+	+	v	+	−
Sorbitol	−	−	−	+	−
Utilization of carbon sources:					
D-Arabitol	−	−	−	+	nd
Citrate	+	+	−	+	nd
Gluconate	−	+	+	v	nd
Inulin	−	−	+	−	nd
Melibiose	+	+	−	+	nd
Palatinose	−	+	+	+	nd
Sorbitol	−	−	−	+	−
D-Turanose	−	d	+	d	nd

[a]For symbols see standard definitions; nd, not determined.

(2) PCR detection with primers ECA1f (5′-CGGCATCATAAAAACACG-3′) and ECA2r (5′-GCACACTTCATCCAGCGA-3′), annealing at 62°C, amplifying a 690-bp DNA fragment (De Boer and Ward, 1995); (3) hybridization with a nonradioactive labeled DNA probe (Helander and Persson, 1996); (4) PCR detection with primers derived from sequences of metalloprotease-coding genes: ERWFOR (5′-ACGCATGAAATCGGCCATGC-3′) T_m 62°C, ATROREV (5′-ATCGATAATTTGATTGTCCT-3′) T_m 52°C, and CHRREV (5′-AGTGCTGCCGTACAGCACGT-3′) T_m 64°C (Smid et al., 1995); (5) detection with a digoxigenin-labeled DNA probe, selected from an

TABLE BXII.γ.244. Additional characteristics of *Pectobacterium carotovorum* subspecies[a]

Characteristic	*P. carotovorum* subsp. *carotovorum*	*P. carotovorum* . subsp. *atrosepticum*	*P. carotovorum* subsp. *betavasculorum*	*P. carotovorum* subsp. *odoriferum*	*P. carotovorum* subsp. *wasabiae*
Indole production	−	−	−	−	−
Growth in 7% NaCl	+	nd	nd	nd	nd
Growth at 40°C	−	−	−	nd	−
Production of aminopeptidase	+	+	+	+	nd
Arginine dihydrolase	−	−	−	−	nd
β-Glucuronidase	nd	nd	nd	−	nd
Lecithinase	−	−	−	−	−
Production of pectolytic enzymes	+	+	+	+	+
Phenylalanine deaminase	−	−	−	nd	−
Phosphatase	−	−	−	−	−
Acid production from:					
Amygdalin	+	+	+	+	nd
L-Arabinose	+	+	+	+	nd
Arbutine	+	+	+	+	nd
Cellobiose	+	+	v	+	nd
Dulcitol	−	−	−	−	nd
Erythritol	−	−	−	−	nd
Esculin	+	+	+	+	nd
DL-Fucose	−	−	−	−	nd
Glycerol	+	+	+	+	nd
Glycogen	−	−	−	−	nd
Lactose	+	+	+	+	+
Malonate	−	−	−	nd	−
Mannitol	+	+	+	+	+
L-Rhamnose	+	+	+	+	nd
D-Ribose	+	+	+	+	nd
Salicin	+	+	+	+	nd
L-Sorbose	−	−	−	−	nd
Starch	−	−	−	−	nd
Sucrose	+	+	+	+	nd
D-Tagatose	−	−	−	−	nd
Trehalose	+	+	+	+	+
Xylitol	−	−	−	−	nd
D-Xylose	+	+	+	+	nd
L-Xylose	−	−	−	−	nd
β-Methylxyloside	−	−	−	−	nd
Utilization of carbon sources:					
Aconitate	nd	nd	nd	−	nd
Adonitol	nd	nd	nd	−	nd
Amygdalin	d	d	+	d	nd
D-Arabinose	−	−	−	−	nd
L-Aspartate	nd	nd	nd	+	nd
Cellobiose	+	+	v	+	+
Dulcitol	nd	nd	nd	−	nd
Erythritol	nd	nd	nd	−	nd
Fucose	nd	nd	nd	−	nd
Methyl-α-galactoside	nd	nd	nd	d	nd
Galacturonic acid	nd	nd	−	+	nd
Gentiobiose	+	+	+	+	nd
α-Methylglucoside	v	+	+	+	nd
N-Acetyl-D-glucosamine	nd	nd	nd	+	nd
L-Glutamate	nd	nd	nd	+	nd
D-Glucosamine	nd	nd	nd	+	nd
Ketogluconate	nd	nd	nd	d	nd
Glucuronate	nd	nd	nd	d	nd
Glutarate	nd	nd	nd	−	nd
DL-Glycerate	nd	nd	nd	+	nd
L-Histamine	nd	nd	nd	−	nd
Hypoxanthin	nd	−	nd	nd	nd
myo-Inositol	+	+	+	+	+
α-Ketoglutarate	+	+	+	+	+
DL-Lactate	nd	nd	nd	−	nd
Lactose	+	+	+	+	nd
Lactulose	nd	nd	nd	+	nd

(*continued*)

TABLE BXII.γ.244. *(cont.)*

Characteristic	*P. carotovorum* subsp. *carotovorum*	*P. carotovorum* subsp. *atrosepticum*	*P. carotovorum* subsp. *betavasculorum*	*P. carotovorum* subsp. *odoriferum*	*P. carotovorum* subsp. *wasabiae*
Malonate	−	−	−	−	−
Maltitol	nd	nd	nd	d	nd
Maltose	v	d	+	d	nd
Maltotriose	nd	nd	nd	−	nd
Mucate	nd	nd	nd	+	nd
Proline	nd	nd	nd	d	nd
Raffinose	+	+	v	+	+
Saccharate	nd	nd	nd	+	nd
L-Serine	nd	nd	nd	+	nd
D-Tagatose	nd	nd	nd	−	nd
Tartrate	−	−	−	−	−
Trehalose	+	+	+	+	nd
Triacetine	−	−	−	−	−
D-Xylose	nd	nd	nd	+	nd
Utilization of nitrogen sources:					
Hypoxanthin	nd	nd	+	nd	nd
Pyrazinamide	nd	nd	+	nd	nd
Sensitivity toward:					
Erythromycin	−	−	−	nd	−
Hypoxanthin	−	nd	nd	nd	nd

[a]For symbols see standard definitions; nd, not determined.

EcoRI digest of a cloned library (Ward and De Boer, 1994).

Several monoclonal antibodies have been produced against *P. carotovorum* subsp. *atrosepticum* strains that can be used for identification and detection in cell suspensions as well as in plant material by (1) ODD (Alarcon et al., 1995); (2) IIF with a sensitivity of up to 240 cells/ml (De Boer and McNaughton, 1987; Gorris et al., 1994; Alarcon et al., 1995); (3) ELISA with a sensitivity of up to 10^3 cells/ml tuber peel extract (Jones et al., 1993; Gorris et al., 1994; Alarcon et al., 1995; Basalp et al., 1995; Hyman et al., 1995; Pérombélon and Hyman, 1995); (4) immunofluorescence (IF) cell staining with a sensitivity of up to 10^5 cells/ml tuber peel extract (Fraaije et al., 1996b); (5) immunofluorescence colony staining (Gorris et al., 1994; Pérombélon and Hyman, 1995; Schober and Van Vuurde, 1997); and (6) slide agglutination test (McLeod and Pérombélon, 1992).

Causes a vascular and parenchymal disease (blackleg) of potato (*Solanum tuberosum*), cauliflower (*Brassica oleraceae* var. *botrytis*), tomato (*Lycopersicon esculentum*), and cabbage (*Brassica oleracea* var. *capitata*), and a storage rot of potato tubers. Bases of stems are blackened, rotted, and slimy. Plants wilt and are stunted or die. The soft rot may affect various parts of the plant, particularly the tubers in storage.

The mol% G + C of the DNA is: 51.3–53.1 (T_m, Bd).

Type strain: ATCC 33260, NCPPB 549, LMG 2386.

GenBank accession number (16S rRNA): Z96090.

c. **Pectobacterium carotovorum** *subsp.* **betavasculorum** (Thomson, Hildebrand and Schroth 1981) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 393) (*Erwinia carotovora* subsp. *betavasculorum* Thomson, Hildebrand and Schroth 1981, 1040.)
be.ta.vas.cu.lo'rum. L. n. *beta* beet; L. n. *vasculum* vascular tissue; M.L. pl. gen. n. *betavasculorum* of the beet's vascular tissues.

Characteristics distinguishing this subspecies from the other *Pectobacterium carotovorum* subspecies are indicated in Table BXII.γ.243. Strain Ecb168 was found to produce an antibiotic that suppresses growth of *P. carotovorum* subsp. *carotovorum* (Costa and Loper, 1994). Causes soft rot and vascular necrosis of sugar beet (*Beta vulgaris*) and, after artificial inoculation, of potato stems.

The mol% G + C of the DNA is: 54.4–54.7 (T_m, Bd).

Type strain: ATCC 43762, NCPPB 2795, LMG 2466.

GenBank accession number (16S rRNA): U80198, Z96091.

d. **Pectobacterium carotovorum** *subsp.* **odoriferum** (Gallois, Samson, Ageron and Grimont 1992) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 394) (*Erwinia carotovora* subsp. *odorifera* Gallois, Samson, Ageron and Grimont 1992, 586.)
o.do.ri'fe.rum. L. masc. adj. *odoriferum* bringing odors, fragrant.

Characteristics distinguishing this subspecies from the other *Pectobacterium carotovorum* subspecies are indicated in Table BXII.γ.243. Grow on nutrient agar, producing colonies that are about 2.5 mm in diameter after 48 h at 25°C and also on yeast peptone dextrose (YPDA) medium (3 g/l yeast extract, 5 g/l peptone, 5 g/l dextrose, 15% agar). Colonies are circular, grayish (to yellowish on media containing sucrose or gelatin), slightly domed, and semitranslucent. Causes odorous soft rot of chicory (*Cichorium intybus* L.). The disease typically results in water-soaked, translucent, wet rot of the etiolated leaves. The development of slimy rot in witloof chicories is always associated with the production of a sweet, ripe, banana-like odor.

The mol% G + C of the DNA is: unknown.

Type strain: CFBP 1878, LMG 17566.

GenBank accession number (16S rRNA): AJ223407.

e. **Pectobacterium carotovorum** *subsp.* **wasabiae** (Goto and Matsumoto 1987) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 393) (*Erwinia carotovora* subsp. *wasabiae* Goto and Matsumoto 1987, 132.)
wa.sa′ bi.a.e. L. gen. n. *wasabiae* of wasabi (*Eutrema wasabi*), name of host plant.

Characteristics distinguishing this subspecies from the other *Pectobacterium carotovorum* subspecies are indicated in Table BXII.γ.243. Colonies on yeast extract-peptone agar plates at 28°C are white, transparent, circular with entire margins, convex, and 1.0–2.0 mm in diameter after 24 h. Growth on yeast extract-peptone agar slants is moderate, white, and butyrous, with high viability. Abundant growth is obtained on yeast extract-peptone agar slants supplemented with glucose or on potato-glucose agar slants, although viability on these media is poor. Causes soft rot and an internal black discoloration on slices of wasabi rhizomes and, after artificial inoculation, also on potato tubers, carrot and radish roots, midribs of chinese cabbage and intact wasabi, and tomato and tobacco plants.

The mol% G + C of the DNA is: 51.4–51.7 (T_m).
Type strain: ATCC 43316, ICMP 9121, LMG 8444.
GenBank accession number (16S rRNA): U80199, AJ223408.

2. **Pectobacterium cacticida** (Alcorn, Orum, Steigerwalt, Foster, Fogleman, and Brenner 1991) Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1[VP] (Effective publication: Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1998a, 394) (*Erwinia cacticida* (Hori 1911) Dye 1969a, 93; *Erwinia cypripedii* Alcorn, Orum, Steigerwalt, Foster, Fogleman, and Brenner 1991, 210.)
cac.ti.ci′ da. Gr. n. *kaktos* prickly plant; Gr. v. *cid* to kill; L. adj. *cacticida* cactus killing.

The characteristics are as described for the genus and as listed in Tables BXII.γ.241 and BXII.γ.242. Colonies grown on PDP (40 g potato dextrose agar (Difco), 5 g agar, 20 g peptone, 1 l distilled water, pH 7.2) for 24 h at 30°C are small, smooth, glistening, circular, entire, slightly convex, ivory, and translucent, and frequently have striations. They produce a distinct odor and become opaque, off-white, and butyrous with age. Causes soft rot of *Opuntia* (cactus) fruits and pads. After artificial inoculation, also causes soft rot of saguaro, organ pipe, senita cacti, tomato fruits, potato slices, and slices of carrot roots.

The mol% G + C of the DNA is: 50.8–51.7 (T_m).
Type strain: ATCC 49481, LMG 17936, ICPB EC186.
GenBank accession number (16S rRNA): AJ223409.

3. **Pectobacterium chrysanthemi** (Burkholder, McFadden and Dimock 1953) Brenner, Steigerwalt, Miklos and Fanning 1973b, 205[AL] emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Erwinia chrysanthemi* Burkholder, McFadden and Dimock 1953, 526.)
chrys.an′ the.mi. M.L. n. *Chrysanthemum* generic name; M.L. gen. n. *chrysanthemi* of chrysanthemums.

The characteristics are as described for the genus and as listed in Tables BXII.γ.241 and BXII.γ.242.

Colonies on potato–glucose agar (pH 6.5) (ATCC culture medium 97) are characteristically umbonate with undulate to coralloid margins ("fried egg") at 3–6 d.

The chrysobactin-mediated iron uptake is essential in enabling the bacterium to systematically attack plants (Expert et al., 1996). Chrysobactin (*N*-(N^2-(2,3-dihydroxybenzoyl)-D-lysyl)-L-serine) is a compound with siderophore activity (Persmark et al., 1989). The iron status is involved in *pel* regulation. Iron acquisition involves an inductive process resulting in differential expression of two siderophore-mediated pathways in relation to external accessibility (Mahe et al., 1995).

The general protein secretory pathway was studied by mutagenesis of cellulase EGZ (Py et al., 1993). *P. chrysanthemi* produces four proteases encoded by *prtA*, *prtB*, *prtC*, and *prtG*, and they are secreted by a type I secretion system encoded by *prtD*, *prtE* (inner membrane), and *prtF* (outer membrane) (Barras et al., 1994). PrtG is an extracellular metalloprotease secreted through a signal peptide-independent secretion pathway. The COOH-terminal exposition of the last four amino acids in the secretion of PrtG plays a key role in the protease secretion pathway (Ghigo and Wandersman, 1994). These metalloproteases are secreted independently of the general export pathway encoded by the *sec* genes. They are secreted via a C-terminal secretion signal and by a secretion apparatus composed of the two inner membrane proteins, PrtD and PrtE, and the outer membrane protein PrtF. PrtD exhibits a secretion signal-regulated ATPase activity (Delepelaire, 1994). PrtD is the ATP-binding cassette (ABC) integral membrane component from the metalloprotease secretion system. PrtA and prtB are two tandem metalloprotease-encoding structural genes (Boyd and Keen, 1993). Further, *P. chrysanthemi* produces two cellulases, encoded by *celZ* (the majority) and *celY* (5% of the total activity), and four types of pectinases. The importance of these various isoenzymes in pathogenicity depends on the plant species infected (Barras et al., 1994). The pectinases are subjected to multiple regulations—KdgR, PecT, PecS, PecM—and products of pectic catabolism encoded by *ogl*, *kduI*, *kduD*, *kdgK*, and *kdgA*. Mutants were isolated, containing genetic fusions of the *kdgK*, encoding 2-keto-3-deoxygluconate (KDG) kinase (Hugouvieux-Cotte-Pattat et al., 1994) or *kdgA* genes to the *lacZ* gene of *Escherichia coli* by infection of a *lacZ* mutant of *E. chrysanthemi* with the phage Mu d(Ap lac) (Hugouvieux-Cotte-Pattat and Robert-Baudouy, 1985). A second set of secondary pectate lyases are preferentially expressed in plants (Barras et al., 1994). The *out* genes are required for the translocation across the outer membrane during secretion of pectate lyases, pectin methyl esterases, exopolygalacturonases, and CelZ. An open reading frame *outT*, located between *outB* and *outC*, has no homology with the *pul* cluster but is involved in secretion. *OutC*, *outD*, and *outE* form an operon, while *outS*, *outB*, and *outT* constitute independent transcription units. *OutT* and the *outCDE* operon are regulated by *kdgRa*, encoding a repressor that negatively regulates the expression of genes involved in pectinolysis and in pectinase secretion. *OutB* and *outS* seem to be expressed constitutively (Ji et al., 1989; Condemine et al., 1992; Nasser et al., 1994).

Mutants defective in secretion of pectinase and cellulase were obtained by chemical and insertion mutagenesis (Andro et al., 1984).

The regulation of pectinolysis was reviewed by Hugouvieux-Cotte-Pattat et al. (1996). Four types of pectinases have been identified: two pectin methyl esterases (PemA, PemB), a polygalacturonase (PehX), nine pectate lyases (PelA, PelB, PelC, PelD, PelE, PelI, PelL, PelZ, PelX), and a pectin acetyl esterase (PaeY) (Shevchik et al., 1997). PecT, encoded by *pecT*, represses the expression of pectate lyase genes *pelC*, *pelD*, *pelE*, *pelL*, and *kdgC*, activates *pelB*, and has no effect on the expression of *pelA* or the pectin methylesterase genes *pemA* and *pemB*. *PecT* activates its own expression (Surgey et al., 1996). *PecS* encodes a repressor that negatively regulates the expression of virulence factors such as pectinases or cellulases (Reverchon et al., 1994; Praillet et al., 1996, 1997). The oligogalacturonate lyase (*ogl*) gene is involved in oligogalacturonides degradation (Reverchon and Robert-Baudouy, 1987) and the *pem* gene encodes the pectin methylesterase (PME) (Laurent et al., 1993). *kduD* mutants altered in pectin degradation were isolated by chemical and Mu d(Ap lac) insertion mutagenesis (Condemine et al., 1986).

Harpins are synthesized in a nutrient-deprived medium and induce plant response, including alkalinization of the apoplastic fluid. Harpin from *P. chrysanthemi* contributes to soft-rot pathogenesis (Bauer et al., 1995). Harpins are encoded by the *hrp* genes, which have at least three biochemical functions: gene regulation, protein secretion, and production of HR elicitor proteins.

P. chrysanthemi strains can be genotypically characterized by RFLP of PCR-amplified fragments of *pel* genes (Nassar et al., 1996a) and ribotyping, which correlates well with the established pathovars (Nassar et al., 1994).

A transducing phage Phi EC2 was described by Resibois et al. (1984) and Phi EC2-resistant mutants, lipopolysaccharide-defective, were isolated by Schoonejans et al. (1987).

Several monoclonal antibodies have been produced against *P. chrysanthemi* strains that can be used for detection or identification by double–antibody sandwich (DAS)-ELISA with a sensitivity of 5×10^3 CFU/ml in carnation stem samples (Nassar et al., 1996b), time-resolved fluoroimmunoassay (TR-FIA) in potato peel extracts with a sensitivity of 10^5 cells/ml (Van De Wolf, 1993).

L-Asparaginase from *P. chrysanthemi* provides an alternative to the enzyme from *E. coli* for the effective treatment of acute lymphoblastic leukemia (Goward et al., 1992). It alters the coagulation system less severely than does *E. coli* asparaginase (Carlsson et al., 1995).

Causes vascular wilts, stunting, soft rots, spotting of leaves or parenchymal necroses of a wide range of plant species and cultivars like *Allium cepa, Capsicum anuum*, *Colocasia esculanta*, *Kalanchoe blossfeldiana*, *Nicotiana tabacum*, *Nopalea* sp., *Pelargonium zonale*, and *Primula* sp. The disease symptoms are usually systemic.

The mol% G + C of the DNA is: 55.1–57.1 (T_m, Bd).

Type strain: ATCC 11663, DSM 4610, LMG 2804, ICMP 5703, NCPPB 402.

GenBank accession number (16S rRNA): U80200, Z96093.

4. **Pectobacterium cypripedii** (Hori 1911) Brenner, Steigerwalt, Miklos and Fanning 1973b, 205^{AL} emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1 (*Erwinia carotovora* biovar cypripedii (Hori 1911) Dye 1969a, 93; *Erwinia cypripedii* (Hori 1911) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171; *Bacillus cypripedii* Hori 1911, 91.)
cyp.ri.ped' i.i. M.L. n. *Cypripedium* generic name; M.L. gen. n. *cypripedii* of cypripedium orchids.

The characteristics are as described for the genus and as listed in Tables BXII.γ.241 and BXII.γ.242. The gene cluster encoding three subunits of membrane-bound gluconate dehydrogenase (GADH) was studied by Yum et al. (1997). Causes a brown rot of cypripedium orchids (*Cypripedium* spp.). The disease usually attacks orchids with fleshy leaves. It starts with small water-soaked lesions that enlarge and become slightly sunken, brownish greasy-looking areas. It may spread down the stem and involve the growing point.

The mol% G + C of the DNA is: 54.1–54.6 (T_m, Bd).

Type strain: ATCC 29267, DSM 3873, ICMP 1591, LMG 2657.

GenBank accession number (16S rRNA): U80201, Z96094.

Genus XXV. ***Candidatus*** **Phlomobacter** *Zreik, Bové and Garnier 1998, 260*

MONIQUE GARNIER

Phlo.mo.bac.ter. Gr. n. *phlomos* bark; Gr. n. *bakterion* a small rod; M.L. neut. *Phlomobacter* rod in the bark.

Filamentous bacteria occurring in the sieve tubes of phloem tissue of plants (Figs. BXII.γ.202 and BXII.γ.203). The original description is from infected strawberry plants (*Fragaria* x *ananassa*) showing leaf marginal chlorosis and stunting in France (Nourrisseau et al., 1993). Like most other phloem-restricted bacteria, *Candidatus* Phlomobacter fragariae has resisted *in vitro* cultivation.

The mol% G + C of the DNA is: not determined.

Type species: *Candidatus* Phlomobacter fragariae Zreik, Bové and Garnier 1998, 260.

FURTHER DESCRIPTIVE INFORMATION

Phylogenetic treatment Phylogenetic analysis of the 16S rRNA gene indicates that *Candidatus* Phlomobacter is a member of the *Enterobacteriaceae* in the *Gammaproteobacteria*. Its closest cultured relative is *Arsenophonus nasoniae*, the recently characterized agent of the son-killer trait in the parasitic wasp *Nasonia vitripennis* (92% 16S rDNA identity) (Fig. BXII.γ.204). The complete sequence of *Candidatus* Phlomobacter 16S rRNA gene is deposited in GenBank under the accession number U91515. The oligo-

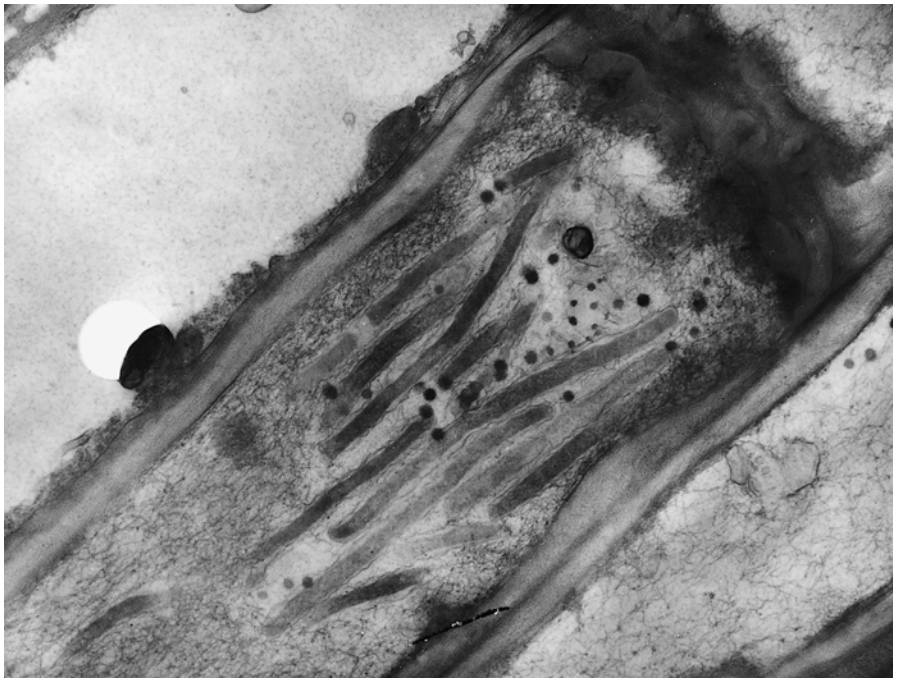


FIGURE BXII.γ.202. *Candidatus* Phlomobacter fragariae cells in a phloem sieve tube of strawberry leaves showing marginal chlorosis (× 20,000).

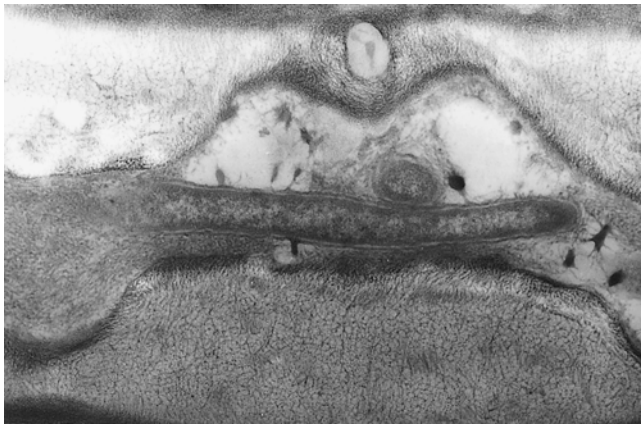


FIGURE BXII.γ.203. *Candidatus* Phlomobacter fragariae cell in a sieve tube with thickened walls (× 29,000).

nucleotide sequence complementary to unique region of the 16S rRNA is 5′-AGCAATTGACATTAGCGA-3′ (Zreik et al., 1998).

Cultivation To date, all attempts to culture *Candidatus* Phlomobacter have failed.

Strain morphology Electron microscopy measurements on thin sections show that *Candidatus* Phlomobacter fragariae cells are 0.2–0.27 μm in diameter and up to 4 μm in length. The cell envelope is 250 nm thick. There is no evidence for flagella or pili (Figs. BXII.γ.202 and BXII.γ.203).

Ecological data, host range *Candidatus* Phlomobacter are phytopathogenic bacteria affecting strawberry (*Fragaria* x *ananassa*) cultivars in which they induce marginal chlorosis of leaf blades, stunting, and fruit malformations. At the ultrastructural level, they induce thickening of the sieve tube walls (Fig. BXII.γ.203). To date, they have not been described in other hosts, and trials to transmit the bacterium via dodder (*Cuscuta campestris*) to periwinkle (*Catharantus roseus*), the experimental plant for phloem-restricted bacteria, have failed. Vector transmission of the bacterium occurs in nature. The insect vector has been identified as *Cixius wagneri* (China). The disease was described in France, and its incidence is highest in strawberry production fields in Southern France. Strawberry nurseries, located in northern France, are not affected. Whether *Candidatus* Phlomobacter fragariae is present in countries other than France has not been investigated.

Procedures for Testing Special Characters

Specific identification *Candidatus* Phlomobacter can be identified by amplification and sequencing of the 16S rDNA. By sequence comparisons, two primers specific for phlomobacteria have been selected on the 16S rDNA sequence. They are efficient and specific for *Candidatus* Phlomobacter detection in plants. When used for DNA amplification in insects, these primers are not specific, as *Candidatus* Phlomobacter shares strong homologies with insect bacterial symbionts and parasites as well as with enterobacteria. Thus, specific identification from hosts other than plants requires sequencing of the 16S rDNA. Genes other than 16S rDNA have been isolated and are under study.

Acknowledgments

Monique Garnier-Semancik died suddenly in May, 2003. She was Director of Research at INRA Bordeaux and served as director of a "Joined Research Unit" in which INRA researchers and University teachers work together. Most of her scientific contributions were to the field of phloem- and xylem-restricted plant pathogenic bacteria, which won her respect both in France and internationally.

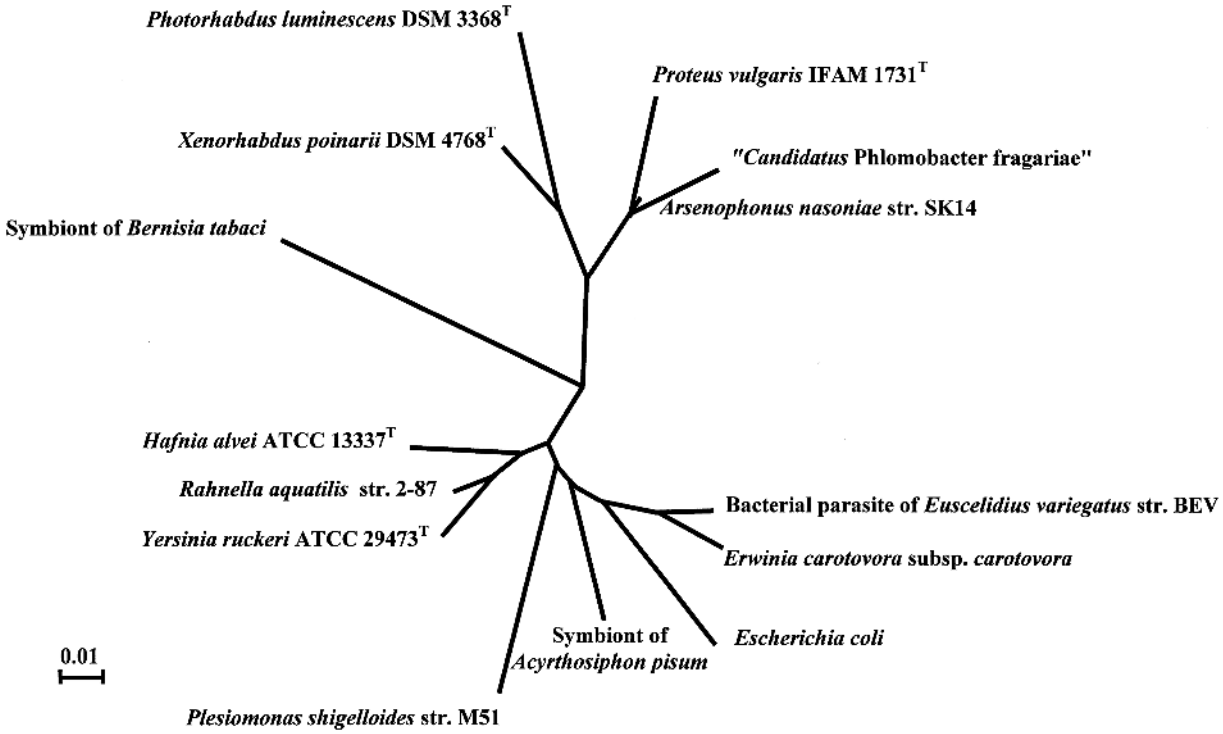


FIGURE BXII.γ.204. Phylogenetic tree constructed with the following 16S rDNA sequences from GenBank using the Suggest Tree Tool of the Ribosomal Database Project (Olsen et al., 1991): *Arsenophonus nasoniae* SK14 (M90801), *Proteus vulgaris* 1731 (X07653), *Hafnia alvei* 13337 (M59155), *Rahnella aquatilis* 2–87 (X79937), *Yersinia ruckeri* 29473 (X75275), *Plesiomonas shigelloides* M61 (M59159), *Acyrthosiphon pisum* symbiont S (M27040), *Escherichia coli* (J01859), parasite of *Euscelidius variegatus* (Z14096), *Erwinia carotovora* (M59149), *Photorhabdus luminescens* 3368 (X82248), *Xenorhabdus poinarii* 4768 (X82253), and symbiont of *Bemisia tabaci* (Z11925). The scale corresponds to 1% substitution. (Reproduced with permission from L. Zreik et al., International Journal of Systematic Bacteriology *48:* 257–261, 1998, ©International Union of Microbiological Societies.)

List of species of the genus Candidatus Phlomobacter

1. *Candidatus* Phlomobacter fragariae Zreik, Bové and Garnier 1998, 260.
fra.ga′riae. L. gen. n. *fragaria, fragariae* of *fragaria*, strawberry.

The description of this organism is identical to that of the genus *Candidatus* Phlomobacter.

The mol% G + C of the DNA is: not determined.
GenBank accession number (16S rRNA): U91515.

Genus XXVI. **Photorhabdus** *Boemare, Akhurst and Mourant 1993, 253VP*

NOËL E. BOEMARE AND RAYMOND J. AKHURST

Pho.to.rhab′dus. Gr. n. *photo* light; Gr. fem. n. *rhabdus* rod; M.L. masc. n. *Photorhabdus* bioluminescent rod-shaped bacterium.

Asporogenous rod-shaped cells 0.5–2 × 1–10 μm. Cell size is highly variable within and between cultures with occasional filaments up to 30 μm long. In the last stage of exponential growth and during stationary growth period, **spheroplasts may occur with an average of 2.6 μm in diameter** (10–20% of cell population), resulting from the partial disintegration of the cell wall. Proteinaceous **protoplasmic inclusions** are synthesized inside a high proportion of cells (50–80%) during the stationary period. Gram negative, motile by means of peritrichous flagella. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Optimum growth temperature usually ~28°C; some strains grow at 37–38°C. Most strains produce pink, red, orange, yellow, or green **pigmented colonies on nutrient agar, and especially on rich media** (tryptic soy agar, egg yolk agar). Bioluminescent, usually detectable by the dark-adapted eye; intensity varies within and between isolates and may only be detectable by photometer or scintillation counter in some isolates; only very few nonluminescent isolates have been reported. **Spontaneous phase shift occurs in subcultures inducing the appearance of phase II clones** characterized by the loss of neutral

red adsorption on MacConkey agar, of production of antibiotics, and of some other properties usually exhibited by wild clones freshly isolated from the natural environment and named phase I variants. Catalase positive. **Do not reduce nitrate.** Negative for oxidase, o-nitrophenyl-β-D-galactopyranoside (ONPG), Voges–Proskauer, arginine dihydrolase, lysine and ornithine decarboxylase tests. Proteolytic for gelatin. Most strains hemolytic for sheep and/or horse blood, some producing an unusual annular hemolysis on sheep blood at 25°C (Fig. BXII.γ.205). Lipolytic activity on Tween 20; many strains lipolytic for Tweens 40, 60, 80, and/or 85. Acid production from glucose without gas. Acid produced from fructose, D-mannose, maltose, ribose, and *N*-acetylglucosamine; weak for acid production from glycerol. Fumarate, glucosamine, L-glutamate, L-malate, L-proline, succinate, and L-tyrosine are utilized as sole carbon and energy sources. Biochemical identification of *Photorhabdus* within the family *Enterobacteriaceae* is summarized in Table BXII.γ.245. Sequence analyses of 16S rDNA show that all *Photorhabdus* strains branch deeply within the radiation of the family *Enterobacteriaceae*, and have **a specific TGAAAG sequence at positions 208–213** (*E. coli* numbering). **The natural habitat for most strains is the intestinal lumen of entomopathogenic nematodes of the genus** *Heterorhabditis* and insects infected by these nematodes. However, some nonsymbiotic strains have been identified as opportunistic pathogens for humans, not nematodes.

The mol% G + C of the DNA is: 43–45.

Type species: **Photorhabdus luminescens** (Thomas and Poinar 1979) Boemare, Akhurst and Mourant 1993, 254 *Xenorhabdus luminescens* Thomas and Poinar 1979, 354.)

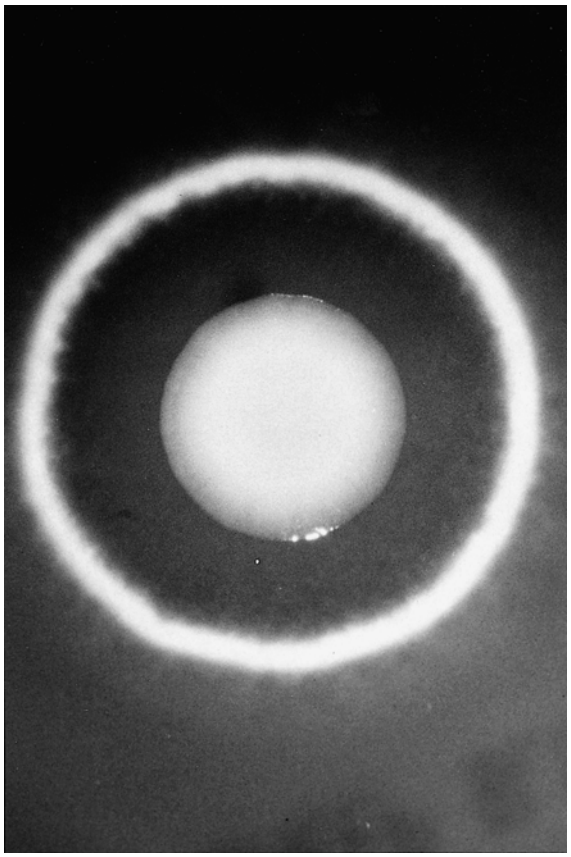


FIGURE BXII.γ.205. Annular hemolysis on sheep blood (10%) agar produced at 25°C by many *Photorhabdus* strains.

Further descriptive information

The strains formerly considered as belonging to *Xenorhabdus luminescens* clearly form a DNA relatedness group that is distinct from all the other *Xenorhabdus* strains (Boemare et al., 1993; Akhurst et al., 1996). These DNA data, together with the significant differences in phenotypic characters between *"X. luminescens"* and the other *Xenorhabdus* species (Akhurst and Boemare, 1988; Boemare and Akhurst, 1988), fatty acids of the whole cellular composition (Janse and Smits, 1990), and chemotaxonomic data (Suzuki et al., 1990), led to the transfer of *X. luminescens* into a new genus, *Photorhabdus*, as *Photorhabdus luminescens* comb. nov. (Boemare et al., 1993). Table BXII.γ.245 lists the characteristics of *Photorhabdus* genus for the three species and four subspecies recognized today (Fischer-Le Saux et al., 1999).

Nevertheless, comparison of 16S rDNA sequences of the type strains of *Photorhabdus* and *Xenorhabdus* species indicate the close phylogenetic relationship of these two genera (Rainey et al., 1995). However, all *Xenorhabdus* strains could be clearly distinguished from strains of *Photorhabdus* by the occurrence of a TTCG sequence at positions 208–211 (*E. coli* numbering) of the 16S rDNA, while *Photorhabdus* have a TGAAAG sequence (Szállás et al., 1997). The nearest phylogenetic neighbor is *Proteus vulgaris* as demonstrated by PCR-RFLP (Brunel et al., 1997) and sequencing of 16S rDNA (Rainey et al., 1995; Suzuki et al., 1996; Szállás et al., 1997; Fischer-Le Saux et al., 1999).

The major cellular fatty acids of *Photorhabdus* are $C_{16:0}$ and $C_{18:1}$; $C_{15:0\ iso}$, $C_{17:0\ iso}$, and $C_{16:1}$ are major components in some strains (Janse and Smits, 1990; Suzuki et al., 1990). Ubiquinone-8 is the respiratory quinone in all strains (Suzuki et al., 1990).

The main difficulty when subculturing *Photorhabdus* strains is the occurrence of a colonial dimorphism that appears suddenly on agar plates. It can be easily detected by two major properties: dye adsorption and antibiotic production (Akhurst, 1980). Each strain occurs as two phase variants that can be described as morphovar, chemovar, and/or biovar. The bacterium isolated from the infective stage (*dauer* larvae) of *Heterorhabditis* was named the phase I variant (Boemare and Akhurst, 1988). Phase I colonies are mucoid and stick to the loop when streaked on plates, produce antibiotic molecules (Akhurst, 1982a), adsorb dyes when incorporated into agar (e.g., the neutral red in MacConkey agar), and are differently pigmented from phase II variants (e.g., red in phase I and yellow in phase II). Among the *Enterobacteriaceae*, cells of some strains of *Photorhabdus* may be the largest known (0.5–2 × 1–10 μm). Phase I cells are larger than phase II cells; they are pleomorphic, comprising rods (80–90%) and spheroplasts (10–20%), and harbor protoplasmic inclusions (Boemare et al., 1983) (Fig. BXII.γ.206). Phase II appears spontaneously during stationary growth period from *in vitro* culture and during nematode rearing on artificial diets. Phase II colonies are not mucoid, do not adsorb dye, and do not produce antibiotics. Several intermediate colony forms, possessing at least some phase I properties, have been recorded (Gerritsen et al., 1992); however, it is not certain that these are not mixtures of phase I and phase II. In general, variation in phase-related characters has been reported qualitatively ("+" and "−"). However, for every character that can be quantified (e.g., luminescence, antibiotic production), it is clear that the difference between phases is a matter of magnitude, not presence/absence. It is highly probable that this holds true for all phase-related characters. Table BXII.γ.246

TABLE BXII.γ.245. Main characteristics of *Photorhabdus* and characteristics differentiating species and subspecies[a]

Characteristic	*Photorhabdus luminescens*[b]	*P. luminescens* subsp. *luminescens*[b]	*P. luminescens* subsp. *laumondii*[c]	*P. luminescens* subsp. *akhurstii*[d]	*Photorhabdus asymbiotica*[e]	*Photorhabdus temperata*[f]
DNA–DNA hybridization with *E. coli*	4%	4%	4%	4%	4%	4%
Mol% G + C of DNA	43–45	43–45	43–45	43–45	43–45	43–45
Maximum growth temperature, °C	35–39	38–39	35–36	38–39	37–38	33–35
Pathogenicity for insects	+	+	+	+	+	+
Motility	+	+	+	+	+	+
Peritrichous flagella	+	+	+	+	+	+
Protoplasmic inclusions	+	+	+	+	−	+
Bioluminescence	+	+	+	+	+	+
Pigmentation	+	+	+	+	+	+
Dye	+	+	+	+	−	+
Antimicrobial production	+	+	+	+	−	+
Oxidation-fermentation	F	F	F	F	F	F
Catalase	+	+	+	+	+	+
Nitrate reduced to nitrite	−	−	−	−	−	−
Oxidase (Kovac's)	−	−	−	−	−	−
Growth in KCN	−	−	−	−	−	−
Indole production	+	+	+	d	−	[−]
Methyl red	−	−	−	−	−	−
Voges–Proskauer	−	−	−	−	−	−
Simmons citrate	d	+	d	d	+ w	d
Hydrogen sulfide production	−	−	−	−	−	−
ONPG (β-galactosidase)	−	−	−	−	−	−
Esculin hydrolysis	+	+	+	+	+	[+]
Urease (Christensen's)	d	−	[+]	d	+	d
Phenylalanine deaminase	[−]	−	d	−	−	[+]
Tryptophan deaminase	[−]	−	d	−	−	[−] w
Amino acid decarboxylases (Moeller's)						
Lysine decarboxylase	−	−	−	−	−	−
Ornithine decarboxylase	−	−	−	−	−	−
Arginine dihydrolase	−	−	−	−	−	−
D-Glucose, acid production	+	+	+	+	+	+
D-Glucose, gas production	−	−	−	−	−	−
Acid production from:						
D-Adonitol	−	−	−	−	−	−
L-Arabinose	−	−	−	−	−	−
Cellobiose	−	−	−	−	−	−
Dulcitol	−	−	−	−	−	−
Fructose	+	+	+	+	+	+
Glycerol	+ w	+ w	+ w	+ w	+ w	+ w
N-acetylglucosamine	+	+	+	+	+	+
myo-Inositol	d	+	[+]	[+]	d w	d w
Lactose	−	−	−	−	−	−
Maltose	+	+	+	+	+	+ w
D-Mannitol	d	d w	−	+	−	[−]
D-Mannose	+	+	+	+	+	+
Melibiose	−	−	−	−	−	−
α-Methyl-D-glucoside	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
L-Rhamnose	−	−	−	−	−	−
Ribose	+	+	+	+	+	+
Salicin	−	−	−	−	−	−
D-Sorbitol	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−
Trehalose	[+] w	+ w	[+] w	[+] w	[+]	[+]
D-Xylose	−	−	−	−	−	−
Utilization of:						
L-Fucose	d	d	−	[+]	−	d
DL-Glycerate	[−]	d	−	−	d	+
L-Histidine	d	+	[+] w	d	d	[+]

(*continued*)

TABLE BXII.γ.245. *(cont.)*

Characteristic	*Photorhabdus luminescens*[b]	*P. luminescens* subsp. *luminescens*[b]	*P. luminescens* subsp. *laumondii*[c]	*P. luminescens* subsp. *akhurstii*[d]	*Photorhabdus asymbiotica*[e]	*Photorhabdus temperata*[f]
myo-Inositol	+	+	+	+	d	[+]
DL-Lactate	[−]	−	−	d w	−	−
D-Mannitol	d	+	−	+	−	[-]
Ribose	+	+	+	+	+	+
L-Tyrosine	+	+	+	+	d	+
Gelatin hydrolysis (Kohn's)	+	+	+	+	+	+
Lecithinase (egg yolk agar)	+	+	+	+	−	+
Lipase (Tween 20)	+	+	+	+	+	+
Lipase (Tween 80)	+	+	+	+	[+]	+
DNase	[−]	−	+	−	−	+
Annular hemolysis at 25°C on:						
Sheep blood agar	d	+	[−]	+	+	+
Horse blood agar	d	+	−	d	+	+

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% are positive; d, 26–75% are positive; [−], 11–25% are positive; −, 0–10% are positive; F, fermentative. The letter w indicates a weak reaction. Data from Akhurst et al. (1996) and Fischer-Le Saux et al. (1999). All tests were done at 28° ± 1°C except annular hemolysis.

[b]Type strain ATCC 29999 isolated from *Heterorhabditis bacteriophora* group Brecon.

[c]Type strain CIP 105565 isolated from *Heterorhabditis bacteriophora* group HP88.

[d]Type strain CIP 105564 isolated from *Heterorhabditis indica*.

[e]Type strain ATCC 43950 isolated from human blood and/or wounds.

[f]Type strain CIP 105563 isolated from *Heterorhabditis megidis* Palaearctic group.

summarizes the characters affected by phase variation. However, both phases show a similar entomopathogenic effect and share all the other bacteriological properties of members of the genus.

In natural conditions, cells of *Photorhabdus* are carried in the intestinal lumen of the free-living stage (infective juvenile L3 or *dauer* larva) of *Heterorhabditis*. The bacterial cells are stored and do not multiply in the gut of the *dauer* host, which is a non-feeding stage. When infective juveniles infect an insect, they release their symbionts into the body cavity of the insect prey. *Photorhabdus* cells multiply inducing toxemia and septicemia and the insect dies. The carcass is a sort of monoxenic microcosm where the symbionts eliminate competitive microorganisms by using several antimicrobial barriers, such as antibiotics possessing a wide spectrum of activity (Akhurst, 1982a) and bacteriocins acting against closely related species (Boemare et al., 1992; Baghdiguian et al., 1993). Nematodes reproduce in the insect carcass, feeding on the insect remains metabolized by the symbiotic bacteria and on the bacterial biomass. When the *dauers* escape the insect cadaver to search for new prey, they carry the symbiont in their gut, ensuring the vertical transmission of the mutualistic association. Although the nematode hosts are the natural vectors of their propagation in the insects, in nutritional terms *Photorhabdus* might be considered entomophilic rather than nematophilic microorganisms.

The life cycle of the clinical strains of *Photorhabdus* is much less certain. *Photorhabdus* has been isolated from five clinical sources in the U.S.A. (Farmer et al., 1989) and six recently in Australia (Peel et al., 1999; Gerrard et al., 2003). Isolations were variously made from tissue, blood, and sputum samples; no definite route of infection has been established. Although some patients may have been immunocompromised, this was definitely not the case for at least two of the Australian patients. These clinical isolates were all easily cultured on standard media at 37°C. They all exhibited the annular hemolysis on sheep blood agar at 25°C. By the time they were identified, all were phase II cultures; it is not known if that is the form in which they were originally isolated.

In vitro subcultures from fresh isolates can be obtained without major difficulties. *Photorhabdus* are mesophilic bacteria able to grow between 15 and 35°C, and some strains at 37–38°C. Subculturing and all biochemical tests should be undertaken around the optimal temperature at 28°C. Usually, nutrient agar or Luria-Bertani agar are sufficient for growth. On minimal media, nicotinic acid, *para*-aminobenzoic acid, proline, tyrosine, and serine are required as growth factors, the mix of growth factors varying between strains (Grimont et al., 1984b). Minimal medium II (BioMérieux) contains all the necessary requirements to test utilization of organic compounds.

Although the shift from phase I to phase II is spontaneous, it is remarkable that the wild *dauer Heterorhabditis* almost exclusively harbor phase I *Photorhabdus* (Akhurst and Boemare, 1990). The role of phase II variants is not clarified, and today we have no convincing data to support a good explanation of their occurrence. Phase change occurs during the *in vitro* stationary period in a highly unpredictable manner (Akhurst and Boemare, 1990). The two phases of *Photorhabdus* differ significantly in respiratory activity (Smigielski et al., 1994). After periods of starvation, phase II cells recommenced growth within 2–4 h after the addition of nutrients, compared with 14 h for phase I cells, indicating a more efficient nutrient uptake ability in the former.

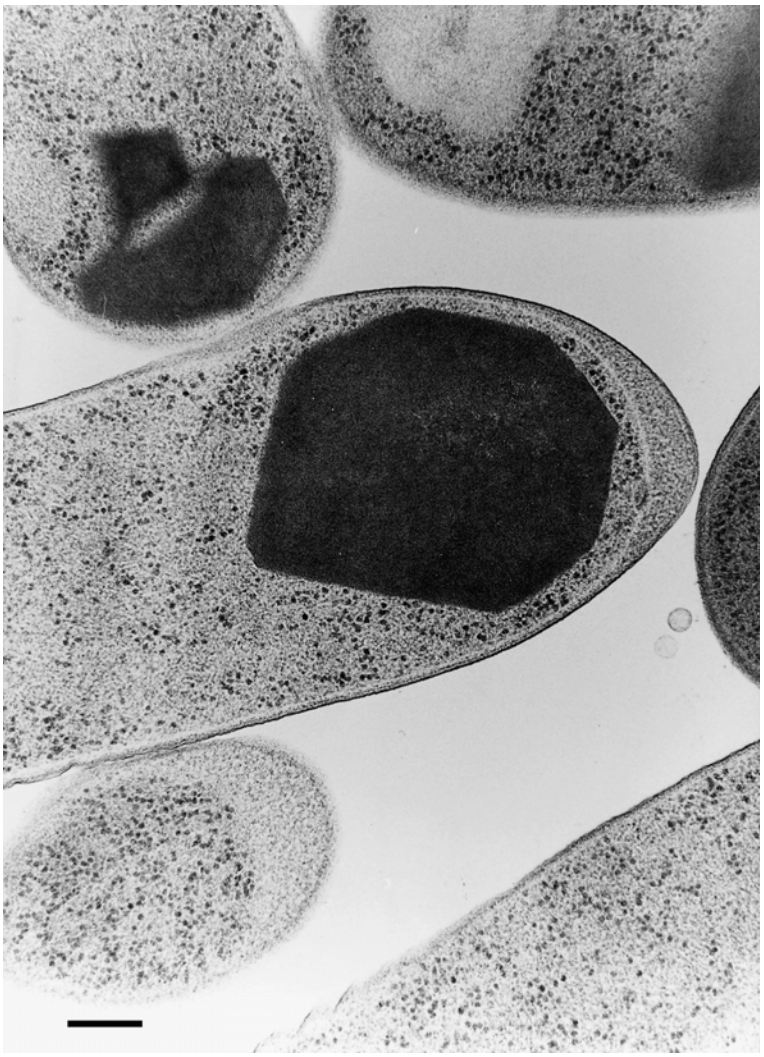


FIGURE BXII.γ.206. Electron micrograph of a fast-freeze fixation and freeze substitution of a culture on nutrient agar in stationary growth of the type strain ATCC 29304 of *Photorhabdus* showing typical inclusions in the protoplasm of the cells. Sections were contrasted with uranyl acetate and lead citrate. Bar = 0.5 μm.

TABLE BXII.γ.246. Differential characteristics of phase variants in *Photorhabdus*[a,b]

Characteristic	Phase I	Phase II
Colonies	mucoid	smooth
Colonial adhesiveness	+	−
Dye adsorption	+	−
Pigmentation	+	+[c]
Protoplasmic inclusions	+	[−] w
Bioluminescence	+	+ w
Antibiotic production	+	−
Lecithinase (egg yolk agar)	+	−

[a]Symbols: +, 90–100% of strains are positive; [−], 11–25% are positive; −, 0–10% are positive. The letter w indicates a weak reaction. All tests were done at 28° ± 1°C.

[b]Characteristics scored in this table are those that always differ between phases, but there are others that differ for the two phases of an individual strain. For those characteristics that can be quantified (e.g., bioluminescence, pigmentation, and protoplasmic inclusions), the difference between phases is a matter of magnitude, not presence/absence. It is highly probable that this holds true for all phase-related characteristics.

[c]Phase II variants of *Photorhabdus* are differently pigmented from phase I variants (e.g., colonies of strain K80 are red in phase I, and yellow in phase II).

Phase II variants may grow a little on complex media previously utilized by phase I variants (Akhurst and Boemare, 1990). However, although reciprocal phase change occurs in *Xenorhabdus*, conversion from phase II *Photorhabdus* to phase I has not been unambiguously demonstrated.

A DNA relatedness study demonstrated unequivocally that phase variation of *Photorhabdus* is a real phenomenon, and not merely an artifact due to contamination, by showing that the two phases of any strain have 100% DNA relatedness. Furthermore, restriction digests and Southern cross analysis of phase I and phase II DNA indicate that the organization of the genome is the same in the two phases (Akhurst et al., 1992).

There are several groups and subgroups within the *Photorhabdus* strains associated with nematodes (*Heterorhabditis* spp.) and human clinical specimens. By using the hydroxyapatite (HA) method to analyze DNA–DNA *Photorhabdus* heteroduplexes, two DNA relatedness groups associated with nematode (*Heterorhabditis* spp.) and another that contains the American human clinical specimens (Farmer et al., 1989) were recognized (Akhurst et al., 1996). More recently, restriction patterns obtained after amplification of the 16S rDNA allowed identification of 12 genotypes

among *Photorhabdus* strains (Fischer-Le Saux et al., 1998). By hybridizing the DNA of some representative strains of each ribosomal genotype, and by using the S1 nuclease method, the previous three genomic groups were confirmed (Fischer-Le Saux et al., 1999). They exhibited between them DNA–DNA hybridization values lower than 42% with ΔT_m higher than 8.7°C. Moreover, the phylogenetic trees inferred from the complete 16S rDNA sequence analysis (neighbor joining, parsimony, and maximum likelihood) delineate the same clusters as both DNA–DNA hybridization methods. Consequently, species and subspecies among *Photorhabdus* were delineated by applying a polyphasic approach, combining 16S rDNA, DNA–DNA hybridization, and phenotypic data (Fischer-Le Saux et al., 1999) (Table BXII.γ.245). Based on DNA–DNA hybridization, 16S rDNA and *gyrB* sequencing, and phenotypic data, the new Australian clinical strains (Peel et al., 1999) will probably constitute a subspecies of *P. asymbiotica* (Akhurst et al., unpublished data). A nonluminescent strain (Akhurst and Boemare, 1986) probably constitutes yet another species (Akhurst et al., 1996).

Within *P. luminescens*, subgroups of strains, which shared very high DNA–DNA hybridization values and ΔT_m lower than 1.5°C and were separated by stable 16S rDNA branching, were identified (Fischer-Le Saux et al., 1999). Consequently, *P. luminescens* was divided into three subspecies (Table BXII.γ.245).

The molecular mechanism of phase variation is uncertain. Lipase and protease are regulated at a posttranslational level in the Irish K122 strain of *P. temperata* (Wang and Dowds, 1993), whereas the *lux* genes are posttranscriptionally regulated in the Hm strain (Hosseini and Nealson, 1995). This contrasts with *X. nematophila* in which the flagellin genes are not transcribed in phase II (Givaudan et al., 1996). These preliminary studies seem to indicate that phase variation in *Photorhabdus* is regulated at a different genetic level than in *Xenorhabdus*.

Several strains of *Photorhabdus* harbor plasmids for which no role or gene has been identified. Heating cultures at 45°C (20 min) and subculturing at 28°C, or mitomycin C treatment, may lead to complete lysis of the cultures, suggesting the occurrence of lysogenic strains (Boemare et al., 1992). Phage tail-like particles, different from those of *Xenorhabdus*, are associated with bacteriocin production in *Photorhabdus* (Baghdiguian et al., 1993).

Antibiograms (Bauer et al., 1966) must be done at 28°C and incubated for 3 days to observe clear zones. *Photorhabdus* have large zones of inhibition around disks impregnated with nalidixic acid, gentamicin, streptomycin, kanamycin, tetracycline, and chloramphenicol, but none around penicillin. Resistance is variable from strain to strain with colistin, ampicillin, carbenicillin, and cephalothin (Farmer, 1984b). It is interesting to note that one patient infected with an isolate sensitive to gentamicin *in vitro* did not respond to gentamicin treatment (Peel et al., 1999).

All strains of *Photorhabdus* have been reported to be entomopathogenic, the LD_{50} usually being <100 cells when injected into hemocoel of the insect *Galleria mellonella* (Farmer et al., 1989; Akhurst and Boemare, 1990; Akhurst and Dunphy, 1993). *Photorhabdus* has also been isolated from human wounds and blood in the U.S.A. (Farmer et al., 1989) and more recently in Australia (Peel et al., 1999). None of the infections was lethal, but some required weeks of treatment.

Photorhabdus strains are the only terrestrial bioluminescent bacteria known today. Their *lux* genes share a strong identity with the *lux* genes of the marine luminescent *Photobacterium*, and *Vibrio* spp. (Frackman et al., 1990). No satisfactory demonstration has yet been provided to explain the role of luminescence in this genus.

Two ecological niches have been identified for *Photorhabdus*: one as a metabolically active form in the insect host, and the other as a quiescent form in the gut of the nonfeeding dauer nematode. The occurrence of nonsymbiotic clinical strains possessing the phase II properties of the nematophilic symbionts and the remarkable differences in respiratory activity between the two phases of the symbiotic strains suggest that there may be a third niche in the soil. However, experiments to test the symbiotic strains ability to grow and survive in external environments indicate that they disappear within a few days (Morgan et al., 1997). *Photorhabdus* strains may enter into a nonculturable but viable survival strategy, as do *Aeromonas*, *Vibrio*, *E. coli*, and *Salmonella* spp.

The specific interaction with the nematode host has been tested by gnotobiological experiments. These assays demonstrate that *Photorhabdus* isolates do not support culture of any *Steinernema* species *in vitro* (Akhurst, 1983b) but in some combinations support culture of nonhost *Heterorhabditis* spp. (Akhurst and Boemare, 1990; Han et al., 1990). Similarly, *Xenorhabdus* spp. do not support culture of *Heterorhabditis* (Akhurst, 1983b). In all the microbial ecological surveys undertaken, a *Xenorhabdus* isolate has never been recovered from *Heterorhabditis* or a *Photorhabdus* isolate from *Steinernema*. Despite the intestinal location of these bacteria, which would allow environmental contamination, the specificity of *Photorhabdus* for *Heterorhabditis* is a remarkable feature of this symbiosis (Boemare et al., 1997a; Forst et al., 1997). When accurate microbial ecology studies are undertaken, using a simple and fast method of PCR-RFLP of 16S RNA (Brunel et al., 1997), a clear correspondence between *Photorhabdus* isolates and nematode species can be seen. From a total of 75 isolates identified in the Caribbean region (Fischer-Le Saux et al., 1998), two genotypes were associated only with *Heterorhabditis bacteriophora* and another two only with *Heterorhabditis indica*.

Photorhabdus–Heterorhabditis and *Xenorhabdus–Steinernema* symbioses are widely divergent. Similar patterns of infectivity, life cycle, and mutualism with nematodes have to be considered to be the result of evolutionary convergence. The symbiotic, pathogenic, and phase variation properties that are the conditions for such associations (Boemare and Akhurst, 1994) do not necessarily imply the same physiological mechanisms. As more genes are sequenced, it will be possible to formulate a clearer picture to explain the convergent evolution of *Photorhabdus–Heterorhabditis* and *Xenorhabdus–Steinernema* symbioses (Boemare and Akhurst, 1994).

Enrichment and Isolation Procedures

Three methods have been used for isolating *Photorhabdus* from nematodes. The "hanging drop" uses a sterile drop of insect hemolymph to which surface-disinfected dauer stage *Heterorhabditis* are added (Poinar and Thomas, 1966). The nematodes exsheath and commence development, releasing their symbiont, which can be subcultured after about 24 h. A second method is to collect under sterile conditions a drop of insect hemolymph from an insect 24 h after infection by *Heterorhabditis*, and to streak it onto nutrient agar. The third method is to crush about 100 surface-disinfected dauer *Heterorhabditis* that have lost their second stage cuticle and to streak the macerate onto nutrient agar (Akhurst, 1980). This latter method is the most rigorous method for assessing the microflora of the intestine of entomopathogenic

nematodes, provided that a suitable control on the effectiveness of the surface disinfection is employed. It reveals the occurrence of *Photorhabdus* in every *Heterorhabditis* sp.

All isolations from nematodes or infected insects should be conducted at or below 28°C.

Human clinical isolates of *Photorhabdus* have been variously obtained from open wounds, tissues aspirated from unerupted lumps, blood, and sputum. These clinical isolates and those from two subspecies of *P. luminescens* can be cultured at 37–38°C and 38–39°C, respectively (Table BXII.γ.245).

Maintenance Procedures

The standard methods of freeze-drying and low temperature storage (liquid nitrogen or at −80°C) used for *Enterobacteriaceae* are also useful for long-term storage of *Photorhabdus* strains; −20°C is unsatisfactory. Cultures do not survive more than a few months in broth or on agar plates at room temperature, and phase variation is likely to occur in this time. Cultures can be routinely maintained for 1 month at 15°C, but storage at 4°C is unsuitable; to prevent phase variation, phase I clones have to be subcultured every week from the neutral red dye-adsorbing clones on MacConkey agar.

Procedures for Testing Special Characters

With the exception of the test for annular hemolysis, which should be conducted at 25°C, all biochemical tests used for phenotypic characterization of *Photorhabdus* should be conducted at 28°C, its optimal growth temperature.

Details of the techniques for identifying the phase variants have been summarized (Boemare et al., 1997b). Adsorption of dyes as described by Akhurst (1980) is the most convenient test to characterize the phase variants. MacConkey agar, or better still MacConkey agar without the bile salts, is a good medium for distinguishing phase I variants (red colonies) from phase II variants (off-white or yellow) (Boemare and Akhurst, 1988). On the NBTA medium [1] described by Akhurst (1980), the adsorption of bromothymol blue by *Photorhabdus* may be confused by the pigmentation of strains and the resulting color of the clones can be difficult to distinguish. As most *Photorhabdus* are pigmented, growth on nutrient agar is often sufficient to differentiate clones of the variants, which differ significantly in not only pigmentation but also colony morphology, with phase I being mucoid and convex whereas phase II is non-mucoid and flattened.

To test antibiotic production by *Photorhabdus*, clones of both variants are spot-inoculated on nutrient agar plates. After growth (generally 48 h), cultures are killed by chloroform vapor (30 min) and covered by fresh nutrient semisolid agar (0.6%) inoculated with a bacterial indicator such as *Micrococcus luteus* (Akhurst, 1982a). The inhibition halos of the indicator culture indicate the phase I variants.

Bioluminescence for most of phase I variants can be checked in a darkroom after 10 min for dark-adaptation of the eyes. To assess absence or weakness of such a light production in the phase II variants, by comparison with the phase I variants' luminescence, a scintillation counter, a fluorimeter, or a photomultiplier have to be used. A loopful of an agar culture of each phase variant culture is suspended in 10 ml of distilled water in a scintillation vial for immediate counting with a fully opened window setting (Grimont et al., 1984b).

1. NBTA medium: nutrient agar containing 0.0025% (w/v) bromothymol blue and 0.004% (w/v) triphenyltetrazolium chloride (Akhurst, 1980).

Differentiation of the genus *Photorhabdus* from other genera

Photorhabdus strains are easily distinguished phenotypically from all the *Xenorhabdus* spp. Bioluminescence and catalase, both physiologically very significant characters, are positive for *Photorhabdus* and negative for *Xenorhabdus*. In most strains, urease is positive and assimilation of DL-lactate is negative for *Photorhabdus* (Table BXII.γ.245); annular hemolysis on sheep blood agar was only observed with *Photorhabdus* strains (Tables BXII.γ.245 and BXII.γ.247). Bioluminescence does not cause confusion with the light-emitting marine bacteria of different families. *Photorhabdus* is differentiated from *Vibrio*, *Alteromonas*, and *Photobacterium* by having peritrichous, not polar, flagella and in not requiring sodium ions for growth.

Taxonomic Comments

By DNA–DNA hybridization *Photorhabdus* is only 4% related to *Escherichia coli*, the type species of the type genus for the family (Table BXII.γ.245). However, *Photorhabdus* possesses the enterobacterial common antigen (Ramia et al., 1982). These data indicate that, while most biochemical tests used for differentiation of the *Enterobacteriaceae* are negative for *Photorhabdus* and although it is only distantly related to the genera that comprise the "core" of the family (Farmer, 1984b), *Photorhabdus* should be retained in the family *Enterobacteriaceae*.

It appears that the genus *Photorhabdus* evolved after the main radiation of *Xenorhabdus* species occurred (Forst et al., 1997) (see Fig. BXII.γ.213 in Genus *Xenorhabdus*). This conclusion, originally derived from the analysis of only seven strains (Rainey et al., 1995), was verified when the 16S rDNA analysis was extended to a balanced number of data sets from *Photorhabdus* and *Xenorhabdus* in comparison to other genera and families (Suzuki et al., 1996; Szállás et al., 1997; Fischer-Le Saux et al., 1999). *Photorhabdus*, *Xenorhabdus*, *Proteus*, and *Arsenophonus* can be considered sister genera because they do not branch to a common ancestor with any other group of the family *Enterobacteriaceae* (Liu et al., 1997a).

Poinar isolated a bacterium from a new nematode, *Heterorhabditis bacteriophora* (Poinar, 1975), which was apparently similar to a *Xenorhabdus* sp. but noticeably bioluminescent (Poinar et al., 1977). Poinar et al. proposed to include this luminescent species in the genus as *X. luminescens* (Thomas and Poinar, 1979). The genus *Photorhabdus* was created in 1993, according to the series of arguments described above, to accommodate this species (Boemare et al., 1993). At the present time, it includes three species, *P. luminescens*, *P. temperata*, and *P. asymbiotica*, one of which contains three subspecies, *P. luminescens* subsp. *luminescens*, *P. lumi-*

TABLE BXII.γ.247. Differential characteristics of *Photorhabdus*, *Xenorhabdus*, and *Proteus*[a]

Characteristic	*Xenorhabdus*	*Photorhabdus*	*Proteus*
Bioluminescence	−	+	−
Catalase	−	+	+
Annular hemolysis on sheep blood agar (25°C)	−	d	−
Urease	−	d	+
Indole	−	d	d
H_2S production	−	−	[+]
Nitrate reductase	−	−	+
Acid from D-mannose	+	+	−

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% are positive; d, 26–75% are positive; −, 0–10% are positive.

nescens subsp. *laumondii*, and *P. luminescens* subsp. *akhurstii* (Fischer-Le Saux et al., 1999). According to the rule 65 (2) of the Code of Nomenclature, generic and subgeneric names that are modern compounds from two or more Latin or Greek words take the gender in the original language of the last component of the compound word. Consequently, *Photorhabdus* genus ending by *rhabdus* (from *rhabdos*, rod in Greek as a feminine word), becomes in modern Latin a feminine word, explaining the feminine names of species and subspecies when they are adjectives (Euzéby and Boemare, 2000). Subspecies names referring to a person take the gender of the person, such as *P. luminescens* subsp. *laumondii* and *P. luminescens* subsp. *akhurstii*.

Further Reading

Akhurst, R.J., R.G. Mourant, L. Baud and N.E. Boemare. 1996. Phenotypic and DNA relatedness between nematode symbionts and clinical strains of the genus *Photorhabdus* (*Enterobacteriaceae*). Int. J. Syst. Bacteriol. *46*: 1034–1041.

Boemare, N.E., R.J. Akhurst and R.G. Mourant. 1993. DNA relatedness between *Xenorhabdus* spp. (*Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. Int. J. Syst. Bacteriol. *43*: 249–255.

Fischer-Le Saux, M., V. Viallard, B. Brunel, P. Normand and N.E. Boemare. 1999. Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. Int. J. Syst. Bacteriol. *49*: 1645–1656.

Forst, S., B. Dowds, N.E. Boemare and E. Stackebrandt. 1997. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annu. Rev. Microbiol. *51*: 47–72.

Szallas, E., C. Koch, A. Fodor, J. Burghardt, O. Buss, A. Szentirmai, K.H. Nealson and E. Stackebrandt. 1997. Phylogenetic evidence for the taxonomic heterogeneity of *Photorhabdus luminescens*. Int. J. Syst. Bacteriol. *47*: 402–407.

List of species of the genus Photorhabdus

1. **Photorhabdus luminescens** (Thomas and Poinar 1979) Boemare, Akhurst and Mourant 1993, 254[VP] (*Xenorhabdus luminescens* Thomas and Poinar 1979, 354.)
lu.mi.nes'cens. M.L. pres. part. *luminescens* luminescing, for its luminescence.

Large rods (2 × 0.5 to 6 × 1.4 μm). Occurs as two phase variants. Luminous, with luminescence more than 100-fold greater in phase I. Maximum growth temperature in nutrient broth occurs at 35–39°C. Indole positive. Weak acid produced from fructose, *N*-acetyl-glucosamine, glucose, glycerol, maltose, D-mannose, ribose, and trehalose by most strains. Some strains acidify D-mannitol. Proteinaceous inclusions in the protoplasm of phase I cells; poorly produced in phase II. The natural habitat is in the intestinal lumen of entomopathogenic nematodes of *Heterorhabditis bacteriophora* (Brecon and HP88 groups), and of *Heterorhabditis indica*. Three subspecies have been described.

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: Hb, ATCC 29999, DSM 3368.
GenBank accession number (16S rRNA): X82248, D78005.

a. **Photorhabdus luminescens** *subsp.* **luminescens** (Thomas and Poinar 1979) Boemare, Akhurst and Mourant 1993, 254[VP] (*Xenorhabdus luminescens* Thomas and Poinar 1979, 354.)

Maximum growth in nutrient broth occurs at 38–39°C. Esculin hydrolysis positive and indole weakly positive. DNase, tryptophan deaminase, and urease negative. Annular hemolysis of sheep and horse blood agars. Does not use DL-lactate as sole source of carbon. D-mannitol used as sole source of carbon and energy. Symbiotically associated with nematodes from the group Brecon of *H. bacteriophora*, the type species of the genus *Heterorhabditis* (Poinar, 1975).

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: Hb, ATCC 29999, DSM 3368.
GenBank accession number (16S rRNA): X82248, D78005.

b. **Photorhabdus luminescens** *subsp.* **akhurstii** Fischer-Le Saux, Viallard, Brunel, Normand and Boemare 1999, 1654[VP]
ak.hurs'ti.i. M.L. gen. n. *akhurstii* of Akhurst, referring to Dr. R. Akhurst, a major contributor to the bacteriological symbionts of entomopathogenic nematodes.

Maximum growth in nutrient broth occurs at 38–39°C. Esculin hydrolysis positive. Tryptophan deaminase and DNase negative. Urease and indole variable. Annular hemolysis observed on sheep blood agar and, in some strains, on horse blood agar. Utilization of DL-lactate as sole source of carbon variable; weak when positive. D-mannitol used and acidified. DL-glycerate utilization negative. Symbiotically associated with the nematode *H. indica* isolated in warm regions; the first strain (strain D1) was isolated from Australia (Darwin, Northern Territory) by Dr. R. Akhurst.

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: FRG04, CIP 105564.
GenBank accession number (16S rRNA): AJ007359.

c. **Photorhabdus luminescens** *subsp.* **laumondii** Fischer-Le Saux, Viallard, Brunel, Normand and Boemare 1999, 1654[VP]
lau.mon'di.i. M.L. gen. n. *laumondii* of Laumond, referring to Dr. C. Laumond, a major contributor to the use of entomopathogenic nematode/bacterial complexes for insect pest control.

Maximum growth in nutrient broth occurs at 35–36°C. Esculin hydrolysis, indole, and DNase positive. Tryptophan deaminase variable and urease mostly positive. Total hemolysis on sheep and horse blood agars; the *Photorhabdus* annular reaction is rare. Does not use L-fucose, DL-glycerate, DL-lactate, or D-mannitol. Symbiotically associated with nematodes of the group HP88 of *H. bacteriophora* isolated in South and North America, southern Europe, and Australia, responding to the satellite DNA probe of the nematode strain HP88 provided by the team of Dr. C. Laumond (Grenier et al., 1996).

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: TT01, CIP 105565.
GenBank accession number (16S rRNA): AJ007404.

2. **Photorhabdus asymbiotica** Fischer-Le Saux, Viallard, Brunel, Normand and Boemare 1999, 1655[VP]
a.sym.bio'ti.ca. Gr. pref. *a* not; M.L. adj. *symbioticus, -a., -um* living together; M.L. fem. adj. *asymbiotica* not symbiotic.

Rods (2 × 0.5 to 3 × 1.0 μm). Maximum growth in nutrient broth occurs at 37–38°C. Yellow or brown pigment. No phase I isolates have been detected, and isolates do not absorb dyes, sometimes weakly produce antibiotics, and are negative for lecithinase on egg yolk agar. Positive for urease, esculin hydrolysis, and for Christensen's citrate, but weakly positive on Simmons' citrate. Tryptophan deaminase negative. Indole and DNase negative. Acid produced from fructose, *N*-acetyl-glucosamine, glucose, maltose, D-mannose, and ribose; weakly produced from glycerol. Proteinaceous inclusions poorly produced. Tween 40 esterase variable. Annular hemolysis on sheep and horse blood agars. Does not use L-fucose, DL-lactate, or D-mannitol. Natural habitat uncertain. All isolates obtained from human clinical specimens.

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: 3265-86, ATCC 439500.
GenBank accession number (16S rRNA): Z76755.

3. **Photorhabdus temperata** Fischer-Le Saux, Viallard, Brunel, Normand and Boemare 1999, 1655[VP]

tem.pe.ra' ta. L. fem. part. adj. *temperata* moderate, because this species grows at moderate temperature.

Large rods (2 × 0.5 to 6 × 1.4 μm). Occurs as two phase variants. Highly luminous. Maximum growth temperature in nutrient broth occurs at 33–35°C. DNase positive. Most of the strains are indole negative. Esculin hydrolysis and phenylalanine deaminase mostly positive. Urease variable. Acid produced from fructose, *N*-acetyl-glucosamine, glucose, D-mannose, and ribose; weak acid production from glycerol and maltose. Proteinaceous inclusions in protoplasm of phase I cells; poorly produced in phase II. Annular hemolysis often occurs on sheep and horse blood agars. Uses DL-glycerate, and does not use DL-lactate as sole source of carbon. D-mannitol is not used by most strains. The natural habitat is in the intestinal lumen of entomopathogenic nematodes of *Heterorhabditis megidis*, of group NC of *H. bacteriophora*, and of *H. zealandica*.

The mol% G + C of the DNA is: 43–45 (Bd).
Type strain: XlNach, CIP 105563.
GenBank accession number (16S rRNA): AJ007405.

Genus XXVII. **Plesiomonas** *Habs and Schubert 1962, 324*[AL]

J. MICHAEL JANDA

Ple.si.o.mo' nas. Gr. masc. n. *plesios* neighbor; fem. n. *monas* unit, monad; M.L. fem. n. *Plesiomonas* neighbor monad (to *Aeromonas*).

Rod-shaped, straight cells, 0.8–1.0 × 3.0 μm (Janda, 1998). Endospores not produced. Gram negative. **Motile.** Growth on common basal agars with most strains growing on mineral media containing ammonium salts and glucose as sole nitrogen and carbon sources (Schubert, 1984). Chemoorganotrophic with both a respiratory- and fermentative-type metabolism. **Facultatively anaerobic. Acid from the catabolism of D-glucose, without gas.** Anaerogenic fermentation of a limited number of carbohydrates **including *m*-inositol**. **Oxidase and catalase positive. Lysine- and ornithine-decarboxylase and arginine dihydrolase positive. NaCl supplementation not required for growth. Most strains susceptible to 10 μg and 150 μg of the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine).** Few extracellular enzymes elaborated (Schubert, 1984). **Starch not hydrolyzed.** 16S rDNA and 16S rRNA analysis indicate plesiomonads belong to the *Gammaproteobacteria*, with maximum homology to members of the *Enterobacteriaceae* (Martinez-Murcia et al., 1992a; Ruimy et al., 1994). Unique genus-specific signature oligonucleotides have not been identified.

The mol% G + C of the DNA is: 51 (Sebald and Véron, 1963).

Type species: **Plesiomonas shigelloides** (Bader 1954) Habs and Schubert 1962, 324 (*Pseudomonas shigelloides* Bader 1954, 455.)

FURTHER DESCRIPTIVE INFORMATION

Contain16S rRNA signature oligonucleotides CUAAUACCG, YCACAYYG (Y = pyrimidine), CUAACUYYG, and UCACACCAUG at positions 170, 315, 510, and 1410 of the *Escherichia coli* numbering system indicative of *Gammaproteobacteria* (Stackebrandt et al., 1988; Martinez-Murcia et al., 1992a). Although previously classified as *Vibrionaceae*, recent phylogenetic data indicates significant evolutionary divergence between *Plesiomonas* and both the *Vibrio/Photobacterium* and *Aeromonas* groups. 5S rRNA, 16S rRNA/rDNA, and 23S rRNA sequence data demonstrate a close phylogenetic relationship between plesiomonads and the *Enterobacteriaceae*, especially the genus *Proteus* (MacDonell et al., 1986; East et al., 1992; Martinez-Murcia et al., 1992a; Ruimy et al., 1994).

P. shigelloides typically appears as short, straight rods without observable curvature. Filamentous bacilli, two to three times the normal cell length, can occur in response to various conditions including exposure to β-lactams (Brenden et al., 1988). Capsules not detected. A heat-labile substance, termed a capsular K or masked antigen by Sakazaki (1984), is produced that inhibits agglutination of cells with homologous O antisera (Farmer et al., 1992). Two types of unsheathed flagella produced. Older cultures express lophotrichous flagellation consisting of a tuft of one to five polar flagella with a wavelength of 3.5–4.0 μm (Ewing et al., 1961; Schubert, 1984). Young cultures (18 h, 25°C) grown on peptone or nutrient based solid media produce peritrichous, curly, flagella with a shorter periodicity of 1.3–1.7 μm (Ewing et al., 1961; Inoue et al., 1991). Up to 68% of young *P. shigelloides* cultures produce lateral flagella when stained with the Flagellar Staining Solution–Shionogi (Inoue and Shimada, 1990; Inoue et al., 1991). Inclusion bodies present in log phase cells consist of polyphosphate granules containing phosphate, potassium, and magnesium (Pastian and Bromel, 1984; Ogawa and Amano, 1987). Laybourn or methylene blue stained inclusions can have bipolar ("safety pin") or central intracellular locations depending on the growth phase (Ogawa and Amano, 1987; Brenden et al., 1988). Intracellular poly-β-hydroxybutyrate granules not formed.

The enterobacterial common antigen (ECA) found exclusively in members of the *Enterobacteriaceae* is present in *Plesiomonas* (Whang et al., 1972; Ramia et al., 1982). ECA appears to be a heteropolymer consisting of *N*-acetylated amino sugars linked to the lipopolysaccharide moiety (Mäkela and Mayer, 1976). Lipid A analysis reveals a common 1,4′-bis-phosphorylated-β-1,6-linked glucosamine backbone with six fatty acid residues either ester-

or amide-linked (Basu et al., 1985). Cellular fatty acid analysis indicates an overall composition similar to other enteric bacteria and *Vibrionaceae* with hexadecanoate ($C_{16:0}$), hexadecenoate ($C_{16:1}$), and octadecanoate ($C_{18:0}$) as major peaks (Lambert et al., 1983; Yamamoto et al., 1991a). Putrescine, cadaverine, and spermidine are the predominant polyamines synthesized (Yamamoto et al., 1991a).

Ultrastructural analysis of *P. shigelloides* reveals a typical cell wall, cytoplasm, and nucleoplasm characteristic of Gram-negative *Bacteria* (Brenden et al., 1988). Electron-dense granules, 50–150 nm in diameter, can be detected as early as 4 h of incubation with maximum size (>500 nm) reached within 12–24 h (Ogawa and Amano, 1987). Electron-transparent vacuoles have also been described (Brenden et al., 1988). Thin section electron micrographs of ruthenium-stained cells demonstrate a string-like acidic mucopolysaccharide material 35 nm thick emanating from the cell surface (Fig. BXII.γ.207). Fimbriae have not been detected.

Plesiomonads produce uniform turbidity in liquid culture without pellicle formation. Autoagglutination rarely observed. Brain heart infusion broth cultures of *P. shigelloides* produce cells with a high surface charge but low hydrophobicity (Abbott et al., 1991). Colonies on nutrient and blood agars are 1.0–2.0 mm in diameter and are smooth, gray, convex, and opaque with an entire edge. Nonhemolytic. Multiple morphovars arising from individual colonies have been reported (Farmer et al., 1992). Cell-associated or diffusible pigments not elaborated; luminescence not observed. Most *Plesiomonas* produce 1.0–2.0 mm flat, colorless colonies on MacConkey, xylose–lysine–desoxycholate, Hektoen enteric, and desoxycholate agars after 24 h growth (Brenden et al., 1988). Delayed lactose fermentation on MacConkey agar by some *P. shigelloides* leads to lactose-positive variants arising upon prolonged incubation, which suggests mixed populations. Salmonella–Shigella, eosin methylene blue, and brilliant green agars are inhibitory to some strains (Brenden et al., 1988).

Growth reported over a wide temperature range (8–45°C); optimal growth occurs at 37–38°C (Schubert, 1984; Miller and Koburger, 1986b). Most plesiomonads multiply in liquid media containing 1–4% NaCl; growth in the presence of 5% NaCl reported to be medium-dependent (Miller and Koburger, 1986b). The pH range for growth has been reported to occur between 4.0–9.0 (Schubert, 1984; Miller and Koburger, 1986b). No growth under extremely acidic (pH 3.0) or alkaline (pH 9.5) conditions.

The genus is biochemically homogeneous. Indole formed. Nitrates reduced. The methyl red test is positive. The Voges–Proskauer test (25°C, 37°C) is negative and acetoin is not produced. No growth in KCN. Citrate, gluconate, malonate, and mucate not utilized; sodium acetate is variable. Phenylalanine deaminase and urease activities absent. The string test (0.5% desoxycholate) is negative. No H_2S produced on triple sugar iron agar slants.

Anaerogenic fermentation of D-glucose, *m*-inositol, maltose, and trehalose. Acid from D-galactose, glycerol, lactose, D-mannose, melibiose, and salicin is variable. No fermentation of adonitol, L-arabinose, cellobiose, dextrin, dulcitol, esculin, α-methyl-D-glucoside, inulin, D-mannitol, melezitose, D-sorbitol, sucrose, L-rhamnose, or D-xylose.

Phosphatase and β-galactosidase (ONPG, O-nitrophenyl-β-D-galactopyranoside) positive. β-D-glucuronidase negative. A number of *P. shigelloides* arylamidases and esterases are detected using semiquantitative micromethods with chromogenic substrates (Manafi and Rotter, 1992). Chitinase elaborated (O'Brien and

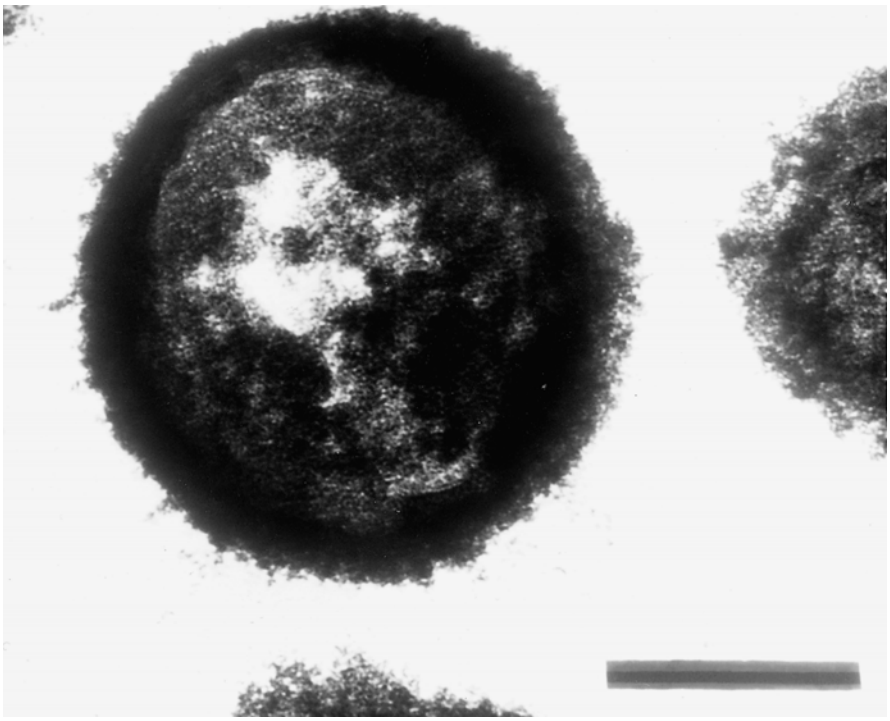


FIGURE BXII.γ.207. Ruthenium-red stained cells of *P. shigelloides* displaying external acidic mucopolysaccharide layer (× 34,000). Bar = 0.5μm. (Reproduced with permission from R.A.Brenden et al., Reviews of Infectious Disease *10:* 303–316, 1988, ©University of Chicago Press.)

Colwell, 1987). Casein, DNA, elastin, fibrinogen, gelatin, polypectate, starch, tyrosine, and Tween 80 are neither degraded nor hydrolyzed.

Formal DNA–DNA hybridization studies on *Plesiomonas* have not been published. Anecdotal information from one epidemiological investigation indicates that all 27 *P. shigelloides* strains constituted a single relatedness group by DNA–DNA hybridization (Holmberg et al., 1986b; Don J. Brenner, personal communication). Between 63 and 100% of *P. shigelloides* strains harbor extrachromosomal elements. Plasmids range in size from 2–8 MDa to very large extrachromosomal elements of 120–312 MDa (Herrington et al., 1987; Ølsvik et al., 1990; Abbott et al., 1991). Plasmid-cured derivatives show loss of expression of β-galactosidase activity (Herrington et al., 1987) and resistance to streptomycin (Marshall et al., 1996). *P. shigelloides* also produces a restriction endonuclease (*Psh* AI) with a novel recognition site of 5′-GACNN/NNGTC (Miyahara et al., 1990). Bacteriocin and phage typing systems have not been described.

An international antigenic scheme recognizes 96 somatic (O) and 48 flagellar (H) antigens of *P. shigelloides* (Aldová and Schubert, 1996). Some serovars show partial or complete identity to *Shigella* somatic antigens. Serovar O17, a common group found in clinical material, reacts with *Shigella* group D antisera (Abbott et al., 1991) and is identical to the O antigen of *S. sonnei* (Sakazaki, 1987). An a, b-a, c type of antigenic relationship also exists between serovar O11 and *S. dysenteriae* 8, serovar O22 and *S. dysenteriae* 7, serovar O23 and *S. boydii* 13, serovar O54 and *S. boydii* 2, and serovar O57 and *S. boydii* 9 (Sakazaki, 1987; Shimada et al., 1994a). Certain plesiomonads share type-specific antigens with *S. flexneri* 6 and common group 1 antigen of *S. dysenteriae* 1 (Albert et al., 1993a). A second unrelated scheme based upon "Schubert" antigens was developed with reference strains from pond water and water insects (Aldová and Schubert, 1996). This scheme is primarily useful in typing environmental isolates; 23 "O" and 5 "H" antigens are recognized.

P. shigelloides is uniformly susceptible *in vitro* to first-, second-, and third-generation cephalosporins, fluoroquinolones, carbapenems, monobactams, and trimethoprim-sulfamethoxazole (Reinhardt and George, 1985; Kain and Kelly, 1989; Clark et al., 1990; Clark, 1992). Widespread resistance to ampicillin, amoxicillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin. Variable results described for tetracycline and aminoglycosides. Most strains produce a β-lactamase using the nitrocefin test (Reinhardt and George, 1985; Clark et al., 1990) but are susceptible to antibiotic-β-lactamase-inhibitor combinations.

The gastrointestinal tract is the primary site from which *P. shigelloides* is recovered. While definitive proof establishing *P. shigelloides* as an enteropathogen is lacking, several lines of evidence support a role in gastroenteritis. These include a low carrier rate, case-controlled investigations, well-circumscribed case reports, and outbreaks of diarrheal disease (Brenden et al., 1988). Carriage rates in asymptomatic individuals range from 0–5.5% with most reported values <0.1% (Arai et al., 1980; Holmberg and Farmer, 1984; Bozsó et al., 1986). Case-controlled investigations have generally found 3- to 20-fold higher isolation rates of plesiomonads from symptomatic patients versus controls (Pitarangsi et al., 1982; Bozsó et al., 1986; Lim et al., 1987). Isolation rates of *P. shigelloides* as high as 22% have been reported from children with bloody diarrhea (Taylor et al., 1986b). Sporadic cases of gastroenteritis have documented high numbers of plesiomonads (3×10^8 CFU/ml) in duodenal biopsies (Penn et al., 1982) and seroconversion to the lipopolysaccharide of infecting strains (van Loon et al., 1989). Two Japanese outbreaks of *P. shigelloides* diarrheal disease caused by serovars O17:H2 and O24:H5 have been associated with contaminated drinking water (Tsukamoto et al., 1978). A third less well-defined outbreak was linked to oyster consumption (Rutala et al., 1982).

P. shigelloides gastroenteritis has been reported around the world. Diarrheal disease can range from a mild enteritis to frank dysentery (Holmberg et al., 1986b; Kain and Kelly, 1989; Rautelin et al., 1995b). Cholera-like illnesses are rare (Sawle et al., 1986). All age groups are affected. Common symptoms include diarrhea with abdominal pain/cramps. Less frequent symptoms include fever, nausea, and vomiting. Risk factors associated with infection include consumption of raw shellfish, foreign travel, and exposure to reptiles and tropical fish (Davis et al., 1978; Van Damme and Vandepitte, 1980; Holmberg et al., 1986b; Tippen et al., 1989).

Extraintestinal disease principally involves septicemia. At least 20 cases have been reported in the literature to date. Most cases of bacteremia involve persons with underlying conditions including neonatality, hematologic dyscrasias, and liver disease (Clark and Janda, 1991; Delforge et al., 1995; Riley et al., 1996). Meningitis accompanying septicemia is primarily a disease of neonates (Billiet et al., 1989). Prolonged membrane rupture prior to delivery predisposes infants to infection (Clark and Janda, 1991). Infrequent extraintestinal complications of *P. shigelloides* infection include biliary disease (Claesson et al., 1984; Körner et al., 1992), pancreatic abscess (Kennedy et al., 1990), pseudoappendicitis (Fischer et al., 1988), arthritis (Gordon et al., 1983), osteomyelitis (Ingram et al., 1987), and ocular infections (Butt et al., 1997a).

Virulence factors are poorly defined (Janda, 1998). Extracellular factors elaborated by select *P. shigelloides* strains include heat-labile and heat-stable enterotoxins (Manorama et al., 1983; Gardner et al., 1987; Matthews et al., 1988) and cytotoxins (Abbott et al., 1991; Albert et al., 1993a). Most strains produce a cell-associated hemolysin that may be responsible for cytotoxic activity (Janda and Abbott, 1993b). Invasion of HeLa and HEp-2 cells has been reported (Binns et al., 1984; Albert et al., 1993a). Sereny (keratoconjunctivitis) test negative. Siderophores not produced (Daskaleros et al., 1991). Human volunteers fed 10^3–10^9 CFU of *P. shigelloides* P012 failed to develop diarrhea (Herrington et al., 1987).

Freshwater environments, including mud and sediment, are the major reservoirs for *P. shigelloides*. A Dutch study (Medema and Schets, 1993) found levels of *P. shigelloides* in surface water samples correlated with fecal pollution (*Escherichia coli*) and the trophic state (chlorophyll *a*, Secchi depth). A subsequent Brazilian investigation did not draw a similar conclusion (de Mondino et al., 1995). A survey of the Suwannee River estuary found most water and sediment samples positive for *P. shigelloides* during three sampling periods (August–February). Flora associated with the ecosystem such as eels, crabs, bream, catfish, crappie, pinfish, clams, and oysters also contained plesiomonads (Miller and Koburger, 1986a). Four of 170 (2.4%) seawater samples were found to contain *P. shigelloides* in one survey (Zakhariev, 1971). Other reported animals found to be colonized with *P. shigelloides* include freshwater fish, tropical fish, dogs, cats, sheep, cows, goats, pigs, monkeys, polecats, turtles, newts, toads, and turkey vultures (Miller and Koburger, 1985; Tippen et al., 1989).

Enrichment and Isolation Procedures

A number of selective media have been designed to recover *Plesiomonas* from environmental samples and contaminated clinical

specimens (e.g., feces). Most of these rely on brilliant green and/or bile salts as inhibitors of competing flora and *m*-inositol, a cyclic polyhydroxyl alcohol, as the differential substrate (von Graevenitz and Bucher, 1983; Farmer et al., 1992). While virtually all *P. shigelloides* are inositol positive, only a limited number of enteric groups (*Enterobacter aerogenes*, *Klebsiella* spp., *Serratia marcescens*) are capable of fermenting this alcoholic sugar.

Inositol-brilliant green-bile salts agar (IBB)[1] has been found to be the best selective media for the recovery of plesiomonads from both clinical (von Graevenitz and Bucher, 1983) and environmental specimens (Miller and Koburger, 1986a). On IBB, *Plesiomonas* colonies appear whitish to pinkish. IBB was 90% sensitive and 100% specific for the recovery of *Plesiomonas* from artificially seeded stool specimens. Optimum *Plesiomonas*/coliform ratios were obtained using IBB (von Graevenitz and Bucher, 1983). However, thermally injured cells may not be satisfactorily recovered using IBB (Miller and Koburger, 1986a). Plesiomonas differential agar (PDA), a variation of IBB, was found superior to IBB for the recovery of *P. shigelloides* in a Bangladesh study (Huq et al., 1991). PDA incubated at 42°C produced optimal results with large colonies (3 mm). Alkaline peptone water (pH 8.6) has been found to be the best enrichment medium for plesiomonads by most investigators (von Graevenitz and Bucher, 1983; Janda, unpublished results), although some studies have found this broth to be unsatisfactory (Millership and Chattopadhyay, 1984).

Maintenance Procedures

Plesiomonas strains are sturdier than *Vibrio* or *Aeromonas* isolates but more fragile than common members of the *Enterobacteriaceae*. Working cultures can be maintained on motility deeps for several months. Permanent stock cultures can be prepared by lyophilization or by freezing cultures at −70°C using cryoprotective agents such as bovine serum albumin (fraction V).

Procedures for Testing Special Characters

For the demonstration of the properties of *Plesiomonas* strains, the same methods are used as those employed for the characterization of *Aeromonas*, *Vibrio*, and members of the *Enterobacteriaceae* (Schubert, 1984).

Differentiation of the genus *Plesiomonas* from other genera

Although *Plesiomonas* has been transferred to the family *Enterobacteriaceae* based upon phylogenetic relatedness, misidentifications as a *Vibrio* or *Aeromonas* can occur by virtue of the production of cytochrome oxidase. Key differential tests that separate these taxa are listed in Table BXII.γ.248.

Taxonomic Comments

Ferguson and Henderson (1947) initially described *P. shigelloides* as a motile *Enterobacteriaceae* culture ("C27") that possessed the major antigen of *Shigella sonnei* phase I. C2 (now ATCC 14030), however, possessed a number of properties distinct from *S. sonnei* including motility, production of indole, delayed lactose fermentation, and the inability to produce acid from D-mannitol. It was therefore placed in the anaerogenic "Paracolon" group by these authors. Because of polar flagellation, Bader (1954) subsequently placed this C27 in the genus *Pseudomonas*, with the epithet *shigelloides* referring to its *Shigella*-like characteristics (Schubert, 1984). Ewing et al. (1961) proposed that this bacterium be transferred to the genus *Aeromonas* based upon a fermentative metabolism and similarities in morphology, flagella arrangement, and biochemical properties. This recommendation was soon replaced by a proposal to create a new genus for *Aeromonas shigelloides*, *Plesiomonas* (Habs and Schubert, 1962). At least two other proposals have subsequently been made to reassign *P. shigelloides* to either the genus *Fergusonia* or *Vibrio* (Vandepitte et al., 1974).

Current phylogenetic and chemotaxonomic data indicate that plesiomonads are more closely related to the *Enterobacteriaceae* than to any other group (Table BXII.γ.249). These data also include proposed 16S rRNA signature nucleotide differences between the *Enterobacteriaceae* and vibrios (Table BXII.γ.250).

Based on phylogenetic evidence a proposal has been made to reassign *P. shigelloides* to the tribe *Proteeae*, considering the 5S rRNA sequence relatedness to *Proteus mirabilis* and *Proteus vulgaris* (MacDonell and Colwell, 1985). However, this proposal seems untenable as classic defining reactions of the tribe *Proteeae* and the genus *Proteus* are entirely absent in *P. shigelloides* (Table BXII.γ.251).

1. Inositol-brilliant green-bile salts agar has the following composition (g/l): proteose peptone (Difco) , 10.0; meat extract (Lab Lemco [Oxoid]), 5.0; NaCl, 5.0; bile salts no. 3 (Difco), 8.5; brilliant green (Merck), 0.00033; neutral red (Merck), 0.025; *meso*-inositol (Merck), 10.0; and agar (Difco), 15.0; pH adjusted to 7.2.

TABLE BXII.γ.248. Key features separating *Plesiomonas*, *Aeromonas*, and *Vibrio*

Characteristic	*Plesiomonas*	*Aeromonas*	*Vibrio*
Growth in nutrient broth/agar (Difco) containing:			
0% NaCl	+	+	D
6% NaCl	−	−	+
O/129 susceptibility:			
10 μg	d	−	D
150 μg	d	−	D
String test	−	−	+
Gas from glucose	−	D	−

TABLE BXII.γ.249. Characteristics resulting in the reassignment of *P. shigelloides*[a,b]

Characteristic	*Plesiomonas*	*Enterobacteriaceae*	*Vibrionaceae*	*Aeromonadaceae*
Possession of ECA	+	+	−	−
Sequence relatedness to **Plesiomonas***:*				
5S rRNA (% homology)	100	96–97	<86	87
16S rRNA (% homology)	100	93–95	91	91
23S rRNA (% homology)	100	91	nd	88

[a]nd, not determined.

[b]Data from MacDonell et al., 1986; Martinez-Murcia et al., 1992a; East et al., 1992.

TABLE BXII.γ.250. Proposed 16S rRNA signature sequence differences[a]

Position(s)	Composition in: *Vibrio*	*E. coli*	*Plesiomonas*
154–167	C–G	U–A	U–A
446–488	U–G	G–C	G–C
614–626	G–C	C–G	C–G
896–903	U–A	C–G	C–G
1123–1149	G–C	U–A	U–A

[a]Adapted from Dorsch et al., 1992; Martinez-Murcia et al., 1992a.

FURTHER READING

Brenden, R.A., M.A. Miller and J.M. Janda. 1988. Clinical disease spectrum and pathogenic factors associated with *Plesiomonas shigelloides* infections in humans. Rev. Infect. Dis. *10*: 303–316.

Holmberg, S.D. and J.J. Farmer III. 1984. *Aeromonas hydrophila* and *Plesiomonas shigelloides* as causes of intestinal infections. Rev. Infect. Dis. *6*: 633–639.

Miller, M.L. and J.A. Koburger. 1985. *Plesiomonas shigelloides*: an opportunistic food and waterborne pathogen. J. Food Prot. *48*: 449–457.

TABLE BXII.γ.251. Defining characteristics of the *Proteeae* and *Plesiomonas*[a]

Characteristic	*Plesiomonas*	*Proteus*	*Providencia*	*Morganella*
Phenylalanine deaminase	−	+	+	+
Swarming motility	−	+	−	−
Urea hydrolysis	−	+	D	+
Degradation of L-tyrosine crystals	−	+	+	+
Pigmentation on DL-tryptophan agar	−	+	+	+

[a]Data from Janda and Abbott (1998a).

List of species of the genus Plesiomonas

1. **Plesiomonas shigelloides** (Bader 1954) Habs and Schubert 1962, 324[AL] (*Pseudomonas shigelloides* Bader 1954, 455.)
shi.gel.loi' des. M.L. fem. n. *Shigella* a generic name; Gr. suffix *eides* similar; M.L. adj. *shigelloides Shigella*-like.

The description of the genus and species are given in Table BXII.γ.252.

Occurs in freshwater and marine environments and inhabitants of those ecosystems including fish, amphibia, and shellfish. Isolated from a variety of animals. Associated with gastroenteritis in healthy adults and children. Occasionally causes systemic infections primarily in persons who are immunocompromised or have underlying illnesses.

The mol% G + C of the DNA is: 51 (Ch).

Type strain: M51, ATCC 14029, CDC 3085-55, DSM 8224, NCIB 9242.

GenBank accession number (16S rRNA): M59159, X74688.

TABLE BXII.γ.252. Characteristics of *Plesiomonas shigelloides*

Test	Reaction or Result
Oxidase	+
Catalase	+
Motility	+
Amino acid decarboxylases (Møller):	
Ornithine decarboxylase	+
Lysine decarboxylase	+
Arginine dihydrolase	+
Methyl red test	+
Voges–Proskauer (25°C, 37°C)	−
Urea hydrolysis	−
ONPG hydrolysis[a]	+
NO_3^- reduced to NO_2^-	+
Utilization of citrate (Simmons)	−
Utilization of malonate, mucate	−
Utilization of acetate	d
Acid production from D-glucose	+
Gas production from D-glucose	−
Acid production from:	
m-Inositol, maltose, trehalose	+
D-Galactose, glycerol, lactose, D-mannose, melibiose, salicin	d
Adonitol, L-arabinose, cellobiose, dextrin, dulcitol, D-mannitol, melezitose, D-sorbitol, sucrose, L-rhamnose, D-xylose	−
Gelatin liquefaction	−
Tyrosine clearing	−
Deoxyribonuclease	−

[a]ONPG, O-nitrophenyl-β-galactopyranoside.

Genus XXVIII. **Pragia** *Aldová, Hausner, Brenner Kocmoud, Schindler, Potužníková, and Petráš 1988a, 187[VP]*

JIRI SCHINDLER, SR.

Pra' gi.a. L. fem. n. *Pragia* of Prague, the city in which strains of the genus were identified.

Gram-negative, oxidase-negative, catalase-positive, peritrichously flagellated rods conforming to the definition of the family *Enterobacteriaceae*. Facultatively anaerobic. Colonies approximately 0.5 mm in diameter are formed on nutrient, Endo, and MacConkey agars. Colonies formed on desoxycholate-citrate agar have a black center. Nonhemolytic on sheep blood agar. A *Shigella*-like odor is produced on nutrient agar. A pigsty-like odor is produced on Endo agar overlaid with a sloping surface of agar. Growth at 4°C, **but not at 42°C**. Optimal growth is obtained at temperatures between 22 and 37°C. Fermentative, forms acid without gas from D-glucose and D-galactose. Motile, reduces nitrates to nitrite, **utilizes citrate, produces hydrogen sulfide, and oxidizes gluconate**. The majority of strains is positive for the methyl red test and delayed positive for acid production from

glycerol and *myo*-inositol. Negative reactions in tests for indole production, Voges–Proskauer test, phenylalanine deaminase, lysine and ornithine decarboxylases, arginine dihydrolase, urease, deoxyribonuclease, malonate, ONPG, growth in KCN, and acid production from L-arabinose, cellobiose, dulcitol, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D-sorbitol, D-sorbose, starch, sucrose, and trehalose. Additional biochemical reactions are given in Table BXII.γ.193 of the chapter on *Enterobacteriaceae*.

The mol% G + C of the DNA is: 46–47.

Type species: **Pragia fontium** Aldová, Hausner, Brenner Kocmoud, Schindler, Potužníková, and Petráš 1988a, 187.

Further descriptive information

Pragia is a member of the *Gammaproteobacteria*. Pragia DNA is 17% or less related to members of all other genera in *Enterobacteriaceae*. Bacteriocin-like agents called fonticins were observed in five *P. fontium* strains. They were active against other *P. fontium* strains as well as against *Budvicia aquatica*, but were not active against *Escherichia coli* or *Shigella sonnei*. Similar bacteriocin-like agents, budvicins, with the same specificity and properties have been isolated from *Budvicia aquatica*. Fonticins have very narrow inhibition zones on indicator strains, are heat sensitive (inactivated at temperatures between 42 and 55°C) and are resistant to trypsin (Šmarda, 1987). They are of corpuscular nature and resemble contracted tails of T4 bacteriophage (Šmarda, 1987). Isolated from fresh water springs or fountains in the South Bohemia region of the Czech Republic (Aldová et al., 1983, 1988b). One human isolate not associated with disease. There is no evidence of pathogenicity for humans or animals.

Differentiation of the genus *Pragia* from other genera

Pragia is easily distinguishable from leminorellae, *B. aquatica*, *C. freundii*, and salmonellae based on whole cell protein analysis (Aldová et al., 1988a; Schindler et al., 1992). Biochemical tests useful in differentiating *Budvicia* from other H_2S-positive genera in *Enterobacteriaceae* are shown in Table BXII.γ.253.

List of species of the genus Pragia

1. **Pragia fontium** Aldová, Hausner, Brenner Kocmoud, Schindler, Potužníková, and Petráš 1988a, 187.[VP]
fon' ti.um. L. gen. pl. n. *fontium* from springs or fountains, the source of isolation of all but one strain.

   The characteristics are as described for the genus and as listed in Table BXII.γ.193 in the chapter on *Enterobacteriaceae*. Occurs in drinking water. One isolate from the stool of a healthy human. No indication of pathogenicity for humans or animals.

   The mol% G + C of the DNA is: 46–47 (T_m).

   Type strain: DRL 20125, HG 16, ATCC 49100, CNTCTC Eb11/82, CCUG 18073, CDC 963-83, DSM 5563.

   GenBank accession number (16S rRNA): AJ233424.

TABLE BXII.γ.253. Differentiation of *Pragia* from other H_2S-positive genera in *Enterobacteriaceae*[a,b]

Characteristic	*Pragia*	*Budvicia*	*Edwardsiella*	*Salmonella*	*Citrobacter*	*Proteus*
Lysine decarboxylase	−	−	+	v	−	−
Ornithine decarboxylase	−	−	+	+	v	v
Urease	−	+	−	−[c]	v	+
ONPG	−	+	−	−	+	−
Indole production	−	−	v	+	v	v
Citrate	+	−	−	+	+	v
Motility	+	v	+[d]	+	+	+
KCN, growth	−	−	−	−[e]	v	+
L-Arabinose, acid	−	v	v	+[f]	+	−
Gluconate oxidation	+	−	−	−	−	−
Growth, 42°C	−	−	+	−	−	−

[a]For symbols, see standard definitions.

[b]Reactions carried out at 37°C, unless otherwise noted.

[c]Subspecies *Salmonella choleraesuis* subsp. *arizonae* and *Salmonella choleraesuis* subsp. *diarizonae* are positive.

[d]Except for *E. ictaluri*.

[e]Subspecies *Salmonella choleraesuis* subsp. *houtenae* is positive.

[f]Except serovars choleraesuis and typhi.

Genus XXIX. **Proteus** *Hauser 1885, 12*[AL]

John L. Penner

Pro' te.us. Gr. n. *Proteus* an ocean god able to change himself into different shapes.

Straight rods, 0.4–0.8 × 1.0–3.0 μm. Gram negative. **Motile** by peritrichous flagella. **Most strains swarm with periodic cycles of migration producing concentric zones, or spread in a uniform film, over moist surfaces solidified with agar or gelatin.** The organisms in this genus conform to the definition of the family *Enterobacteriaceae*. **They are facultatively anaerobic, chemoorganotrophic, having both a respiratory and a fermentative type of metabolism.** Optimal growth temperature is 37°C. **Oxidase negative; catalase positive. Methyl red positive; species vary in indole production, Voges–Proskauer, and Simmons citrate tests. They oxidatively deaminate phenylalanine and tryptophan. Urea is hydrolyzed. Lysine decarboxylase negative and arginine dihydrolase negative; only *Proteus mirabilis* decarboxylates ornithine. All but *Proteus myxofaciens* decompose tyrosine to produce a clearing on**

agar media in which the insoluble amino acid is incorporated. Grow on KCN. H_2S is usually produced. Malonate is not utilized. D-glucose and a few other carbohydrates are catabolized with production of acid and usually gas. Does not produce acid from inositol or from straight chain tetra-, penta-, or hexahydroxy—alcohols, but generally produces acid from glycerol. One or more species ferment maltose, sucrose, trehalose, and D-xylose. Human pathogens, causing urinary tract infections; also are secondary invaders, causing septic lesions at other sites of the body. Occurs in the intestines of humans and a wide variety of animals; also occurs in manure, soil, and polluted waters. *P. myxofaciens* has been isolated only from gypsy moth larvae. Based on 16S rDNA sequence analysis, *Proteus* belongs to the family *Enterobacteriaceae* within the *Proteobacteria* (Woese et al., 1985; Niebel et al., 1987; Stackebrandt et al., 1988).

The mol% G + C of the DNA is: 38–41 (Falkow et al., 1962).

Type species: **Proteus vulgaris** Hauser 1885, 12 emend. Brenner, Hickman-Brenner, Holmes, Hawkey, Penner, Grimont and O'Hara 1995, 870.

Further descriptive information

Comparative analysis of 16S ribosomal RNA sequences (the oligonucleotide cataloging method) showed that *P. mirabilis* belongs to the *Gammaproteobacteria*, which includes the family *Enterobacteriaceae* (Woese et al., 1985; Stackebrandt et al., 1988). DNA relatedness studies have shown that members of the genus *Proteus* are only distantly related to the other species in the family *Enterobacteriaceae* and from an evolutionary standpoint they are therefore at the periphery of this family (Brenner et al., 1978). *P. mirabilis* strains are a highly related group. They are 55% related to *P. vulgaris* and to *P. penneri* strains. *P. penneri* consists of a highly related group of indole-negative strains that ferment salicin and hydrolyze esculin. They were classified as *P. vulgaris* biogroup 1 (Brenner et al., 1978) before they were recognized as a new species (Hickman et al., 1982c). At present, *P. vulgaris* consists of two biogroups. *P. vulgaris* biogroup 2 consists of strains that are indole positive, that ferment salicin and hydrolyze esculin. Strains of this biogroup conform to the description of the genus and include the vast majority of the *P. vulgaris* strains isolated from clinical sources. Computerized analysis of electrophoretic protein patterns and DNA relatedness tests showed that *P. vulgaris* biogroup 3 strains (indole positive, salicin negative, and esculin negative) are a heterogeneous group that includes four genomospecies, one of which includes the type strain (Costas et al., 1993; Brenner et al., 1995). Future studies will likely lead to the identification of new species within this biogroup.

On media solidified with gelatin or agar, the bacteria migrate from the point of inoculation to spread over the surface of the medium. The phenomenon, known as swarming, is attributed to the dimorphic nature of the bacteria. The short mononucleoid, peritrichously flagellated rods that occur singly in liquid media, referred to as swimmers, differentiate when transferred to solid media to produce long, nonseptate, multinucleoid swarmers with large increases in the number of flagella (Belas, 1992). Glutamine has been identified as the chemoattractant that signals the surface-induced differentiation (Allison et al., 1993). Differentiation into swarmer cells is associated with marked increases in synthesis of lipopolysaccharide with long O-polymers and a higher fluidity of the outer membrane (Sidorczyk and Zych, 1986). The production also of extracellular slime forming a thin cocoon covering swarms of cells (VanderMolen and Williams, 1977) or capsular polysaccharide containing galacturonic acid and *N*-acetylgalactosamine facilitates migration of swarmer cells (Stahl et al., 1983; Gygi et al., 1995). Swarmer cells are the virulent forms of *Proteus* and during differentiation a coordinated increase occurs in the synthesis of urease, hemolysins, and proteases (Jin and Murray, 1987; Allison et al., 1992, 1994).

Three phases have been recognized in the process of swarming. In the first phase, differentiation of swimmer cells into swarmer cells takes place. In the second phase, groups of swarmer cells migrate from the point of inoculation and continue to migrate until either a change in direction occurs or the number of swarmers is reduced by a loss of some along the way. In the third phase, migration ceases, which results in consolidation. At this point, the swarmer cells form septa to become short cells that multiply for a period of time before producing another generation of swarmer cells (Williams and Schwarzhoff, 1978; Rózalski et al., 1997). Cyclic repetitions of the phases leads to concentric rings surrounding the site of inoculation. Some strains (or variants) produce a uniform film (referred to as spreading) without periodic cycles (C variant of Belyavin, 1951; Z variant of Coetzee and Sacks, 1960). Some strains neither spread nor swarm and merely form distinct colonies.

The swarming of *Proteus* makes it difficult to isolate bacteria of other species from pathological specimens plated on agar media and to prevent swarming; inhibitors such as bile salts and detergents are incorporated in the media (Kopp et al., 1966). The compound *p*-nitrophenyl glycerol, at concentrations of 0.1–0.3 mM, inhibits swarming without affecting flagellation or motility, and because of its low toxicity to *Proteus* and other bacteria, its use has been advocated (Williams, 1973). With continued incubation, however, the *Proteus* strains eventually swarm in the presence of this compound and thus isolations of *Campylobacter* species and other bacteria that require longer periods of incubation require other measures such as the incorporation of antibiotics in the isolation media (Firehammer, 1987). The incorporation of charcoal in the medium has been reported to suppress swarming without interfering with the growth of other bacterial species (Alwen and Smith, 1967). Other methods of preventing swarming include reduction of the salt concentration of the media and increasing the concentration of agar to 4% (New Zealand agar) or 7% (Japanese agar).

In the Dienes test, swarms of two strains that do not penetrate into each other but form a sharp line between them indicate that the strains are different but strains that produce swarms that merge into each other without a line of demarcation are interpreted to be the same (Dienes, 1946). This test has been used in epidemiological investigations of *Proteus* infections, mostly of *P. mirabilis*, to determine identity and nonidentity of clinical isolates (Story, 1954; Skirrow, 1969). However, strains of different biovars have been found to swarm into each other (Kippax, 1957), and, in some cases, results obtained with the test fail to correlate with bacteriophage typing results (Hickman and Farmer, 1976). The production of the demarcation line is apparently unrelated to the flagellar (H) antigens (Sourek, 1968; Skirrow, 1969) but appears to be dependent on both the bacteriocins produced by the swarming strains and the bacteriocins to which they are susceptible (Senior, 1977a). The Dienes test is useful in combination with other procedures for epidemiological typing and when its limitations are recognized.

A well-known distinguishing feature of the genus is the production of urease. It is an important taxonomic criterion that differentiates the genus from most other species of *Enterobacteriaceae* except for the genera *Morganella* and *Providencia*, some

species of which also produce the enzyme. The enzyme is constitutive in most strains of *P. mirabilis* and inducible in *Proteus vulgaris*, *Proteus penneri*, and some *P. mirabilis* strains (Mobley et al., 1987; 1995; McLean et al., 1988; Mobley and Hausinger, 1989). The enzyme is a 212–280 kDa protein consisting of three subunits (Jones and Mobley, 1989; Mobley and Hausinger, 1989) and is known to have an important role in the development of human urinary tract infections (Mobley, 1996).

An important distinguishing feature of *Proteus*, *Providencia*, and *Morganella* is their ability to oxidatively deaminate a variety of amino acids, producing keto acids and ammonia (Bernheim et al., 1935; Stumpf and Green, 1944; Singer and Volcani, 1955). The carboxylic acids bind iron and have siderophore activity (Evanylo et al., 1984; Drechsel et al., 1993). Addition of ferric chloride solution to keto acids in aqueous solution produces different colors dependent upon the amino acid from which the keto acid was produced (Singer and Volcani, 1955), and the same colors are produced when ferric chloride solution is added to bacteria grown on nutrient media supplemented with the amino acids. This is the basis of the diagnostic test used in the clinical laboratory to distinguish these three genera from other *Enterobacteriaceae*. Tests for phenylalanine deaminase and tryptophan deaminase are widely used (Henriksen, 1950; Thibault and Le Minor, 1957). The gene encoding an amino acid deaminase (*aad*) in *P. mirabilis* has been identified (Massad et al., 1995). The probe for the *aad* gene does not hybridize with DNA from *Providencia* and *Morganella* strains, suggesting that the deaminases of these three genera are unrelated. As with ureases, an end product of the deaminases is ammonia, which is now known to have an important role in pathogenesis by elevating pH, but the contributions of the deaminases in the pathogenic process have yet to be determined (Rózalski et al., 1997).

The production of amines by *Proteus* species and *Morganella morganii* when grown in nutrient broth is a distinguishing biochemical property of the two genera (Proom and Woiwod, 1951). The inability of *Providencia rettgeri*, classified during that period as *Proteus rettgeri*, to produce amines under these conditions was cited as an important criterion in their proposal for removing this species from the genus *Proteus*. Simplified tests to detect amines for differentiation of these genera from other *Enterobacteriaceae* in the clinical laboratory have not been developed. However, the amines continue to be of interest, particularly with the finding that a carcinogenic nitrosamine (*N*-nitrosodimethylamine) is produced in urine by *P. mirabilis* during the course of a urinary tract infection (Brooks et al., 1972).

The antigens of the genus *Proteus* that are used for differentiating the serovars are the lipopolysaccharide (O) antigens, the flagellar (H) antigens, and the capsular (K or C) antigens. The O antigenic scheme of Kauffmann and Perch for *P. vulgaris* and *P. mirabilis* (Kauffmann, 1966) lists 49 serovars. Tube agglutination tests and cell suspensions boiled for 1 h were used to determine the O specificities. Additional serovars have been identified that are not included in the scheme of Kauffmann and Perch (Larsson and Olling, 1977; Penner and Hennessy, 1980).

Seventeen serovars of the Kauffmann and Perch scheme were found to include only *P. vulgaris* strains, 27 included only *P. mirabilis* strains, and five included strains of both species. Since isolates generally agglutinate in antisera against the same species, separation of the serovars to provide individual schemes for each species facilitated routine serotyping (Penner and Hennessy, 1980). In the system developed by Penner and Hennessy for serotyping *P. vulgaris* and *P. mirabilis*, the passive (indirect) hemagglutination technique was employed to determine the specificities of soluble heat extracted antigens. Their system included 22 *P. vulgaris* and 32 *P. mirabilis* strains from the Kauffmann and Perch scheme, five new serovars of *P. vulgaris*, and six new serovars of *P. mirabilis*. However, from the large numbers of untypeable smooth strains isolated in epidemiological studies, it is clear that a substantial number of serovars remain undefined (Larsson and Olling, 1977; Larsson, 1980, 1984; Penner and Hennessy, 1980). Reports differ on the distributions of serotypes of *P. mirabilis*, but among those listed the most frequently occurring are isolates of O serotypes 3, 6, 10, 27, and 28 (Lányi, 1956; de Louvois, 1969; Larsson and Olling, 1977; Penner and Hennessy, 1980; Rózalski et al., 1997). In these earlier studies the strains of *P. vulgaris* were not differentiated into biogroups and thus the collections included a few biogroup 1 strains that would now be classified as *P. penneri* (Hickman et al., 1982c). A system for serotyping *P. penneri* has not been developed. However, one serotypically homogeneous group of strains collected from three countries has been identified and designated group O61 (Sidorczyk et al., 1996). The molecular structures of the lipopolysaccharides of at least 10 different strains of *P. penneri* have been determined (Zych et al., 1997). Examination of the variety of structures suggests the possibility that more serotypes will be defined in the future.

Much research has recently focused on the structures of the lipopolysaccharide (LPS) of *P. vulgaris*, *P. mirabilis*, and *P. penneri*, and progress in this area has been the subject of a major review (Rózalski et al., 1997). The structure of lipid A is comparable to the lipid A of *Escherichia coli* and salmonellae but differs in its fatty acid composition and in the presence of a 4-deoxy-arabinosyl residue that substitutes the phosphate residue of glucosamine (Sidorczyk et al., 1983). The biological activity of *Proteus* lipid A is comparable to that of *E. coli* and salmonellae (Sidorczyk et al., 1978). The core oligosaccharides of *P. vulgaris* and *P. mirabilis* consist of Kdo (3-deoxy-D-*manno*-octulosonic acid), heptoses, and hexoses, as in *E. coli* and salmonellae, but differ in that they have terminal or chain-linked galacturonic acids that contribute significantly to the immunospecificity of the oligosaccharide (Kotelko, 1986; Sidorczyk et al., 1987). The O-polysaccharide polymers consist of repeating units composed of hexoses, hexosamines, 6-deoxy sugars, uronic acids with amino acids attached to their carboxyl groups, and other noncarbohydrate acidic components (Sidorczyk et al., 1975; Kotelko, 1986; Sidorczyk et al., 1995).

P. vulgaris strains OX19 and OX2 and *P. mirabilis* strain OXK are the test strains for O antigens 1, 2, and 3, respectively, in the antigenic scheme of Kauffmann and Perch (Kauffmann, 1966). Antibodies in sera from patients with rickettsial infections agglutinate either one or two of these strains. This reaction is the basis of the Weil-Felix test, which is a presumptive test for rickettsial infections. Sera from patients with endemic or epidemic typhus agglutinate the OX19 strain, patients infected with the spotted fever group of rickettsiae agglutinate both OX19 and OX2 strains, and patients with scrub typhus agglutinate the OXK strain. The reactions have been suspected to be due to LPS and the finding that sera from patients with spotted fever react with LPS from both the rickettsial strain and the *P. vulgaris* OX2 strain indicates this to be the case (Amano et al., 1993). Since *Proteus* infections may also stimulate antibody production and *P. mirabilis* strains with the O3 antigen are among the most frequently isolated, positive Weil-Felix tests should be interpreted with caution. Furthermore, the heterophile antigens to which the cross-reactions in the Weil-Felix test are attributed also occur in *Legionella*

bozemanii and *Legionella micdadei*, indicating that these antigenic specificities are more widely distributed than originally believed (Sompolinsky et al., 1986; Westfall et al., 1986).

Analysis of the flagellar (H) antigens of *P. vulgaris* and *P. mirabilis* was carried out by Perch (1948). A total of 19 were identified and included in the Kauffmann and Perch antigenic scheme (Kauffmann, 1966). Cross-reactions among the H antigens are numerous and complex. Five antigenic factors were identified in the H 1 complex and other H antigens showed even greater complexity. The use of H antigens for routine serotyping of *P. vulgaris* and *P. mirabilis* strains has not been adopted and has been limited essentially to the initial studies of Kauffmann and Perch (Perch, 1948). The flagellar antigens of *P. penneri* have not been investigated.

Capsular antigens, unlike the K antigens of other *Enterobacteriaceae*, were reported to be present in the genus *Proteus* (Namioka and Sakazaki, 1959). These antigens, designated C antigens to distinguish them from the *E. coli* L and B antigens, inhibit O agglutination, retain antibody binding capacity after 1 h at 100°C, and have antigenic specificities restricted to certain O groups. The authors suggested that numerous serological types of C antigens occur in the genus *Proteus*, but a system for identifying them has not been developed. Research has focused on the role of capsules in facilitating swarming (Stahl et al., 1983), and a polysaccharide that evidently has this effect has been identified and chemically characterized (Gygi et al., 1995). Investigations are also under way to examine the role of polysaccharide capsules or glycocalyces in the formation of biofilms during infections of the urinary tract. It has been hypothesized that the biofilm is a major virulence factor because it enhances bacterial adhesion, surrounds the bacteria to protect them from the immune response and from exposure to antibiotics, and provides a microenvironment in the kidney and bladder that is conducive to stone formation (Nickel et al., 1985; McLean et al., 1988; Clapham et al., 1990).

Since the last edition of the *Manual*, numerous investigations of the surface structures of *P. vulgaris*, *P. mirabilis*, and *P. penneri* have resulted in clarification of the nature of the well-known hemagglutinins and fimbriae and in the discovery of several new types of fimbriae. A greater understanding of the mechanisms of pathogenesis has been gained. The MR/P (mannose resistant *Proteus*-like) fimbriae, also known as type IV fimbriae, have been the most extensively studied. These are highly immunogenic proteins 7 nm in diameter. The MR/P fimbriae have been isolated and purified and the genes involved in their determination have been identified (Sareneva et al., 1990; Bahrani et al., 1991; Bahrani and Mobley, 1993, 1994). Evidence obtained in experiments using the mouse model indicates that these fimbriae are associated with colonization of the upper regions of the urinary tract (Bahrani et al., 1994).

The MR/K (mannose resistant, *Klebsiella*-like) type 3 hemagglutinins are thin (diameter of 4–5 nm) and hemagglutinate tanned erythrocytes. Two antigenic types have been found but neither type is antigenically related to *Klebsiella* fimbriae (Old and Adegbola, 1982). MR/K hemagglutinins are more characteristic of *P. penneri* than of *P. mirabilis* (Yakubu et al., 1989). *Proteus* strains with MR/K hemagglutinins have a high affinity for the materials of which catheters are made, and this feature has been cited as a factor in prolonged chronic urinary tract infections of catheterized patients (Mobley et al., 1988; Yakubu et al., 1989; Roberts et al., 1990).

Of the newly discovered fimbriae, one type designated PMF for *P. mirabilis* fimbriae, has been suggested to be important in the colonization of the bladder, but not the kidney (Bahrani et al., 1993; Massad et al., 1994b). Another type, referred to as ambient temperature fimbriae (ATF) of *P. mirabilis*, is optimally produced at 23°C and apparently does not have a role in pathogenesis (Massad et al., 1994a). A third type, also expressed by *P. mirabilis*, was referred to as nonagglutinating fimbriae (NAF) (Tolson et al., 1995). It does not have the hemagglutinating properties of MR/P or MR/K fimbriae, but adheres to uroepithelial cells, indicating possible importance as a virulence factor in establishing urinary tract infections.

The ability of *Proteus* species to produce cytotoxic hemolysins has been known since 1919 (Wenner and Rettger, 1919), but definitive studies had to await the development of molecular biological technologies. With the aid of new techniques, it has been established that *Proteus* species produce two distinct hemolysins (Welch, 1987). The HlyA hemolysin is genetically related to the α-hemolysin of *E. coli*, has a molecular size of 110 kDa, and is a Ca^{2+}-dependent, pore-forming cytotoxin (Koronakis et al., 1987, Welch, 1991). The HpmA hemolysin (166 kDa) is related to the ShlA hemolysin of *Serratia marcescens* and is calcium independent (Uphoff and Welch, 1990). A protein, HpmB (63 kDa), which is closely related to the ShlB protein of *S. marcescens* is responsible for the transport and activation of the HpmA protein (Swihart and Welch, 1990b; Uphoff and Welch, 1990). Nearly all the *P. vulgaris* and *P. mirabilis* strains produce the HpmA hemolysin, but only a few *P. vulgaris* produce the HlyA hemolysin (Swihart and Welch, 1990a). Most strains of *P. penneri* produce the HpmA hemolysin and most also produce the HlyA hemolysin (Rózalski and Kotelko, 1987; Lukomski et al., 1990; Senior, 1993). Expression of hemolysins is associated with increased cytotoxicity, cell invasiveness, and a lower LD_{50} in mice, but is not associated with increased kidney colonization or histopathological changes in murine urinary tract infections (Peerbooms et al., 1983, 1984, 1985; Mobley and Chippendale, 1990).

Proteus spp. are among the few Gram-negative species capable of producing proteolytic enzymes that degrade immunoglobulins. Strains of *P. vulgaris*, *P. mirabilis*, and *P. penneri* have been shown to produce immunoglobulin A (IgA) proteases (Senior et al., 1988; Wassif et al., 1995). Analysis of the proteolytic activity of one *P. mirabilis* strain isolated from a patient with urinary tract infection showed that it has a much wider range of activity than the usual IgA proteases. Both serum and secretory IgA1 and IgA2, both the free and IgA-bound secretory component, and IgG are susceptible targets of this enzyme but its role as a virulence factor in urinary tract infections remains uncertain (Loomes et al., 1990). A polypeptide with protease activity that is secreted by uropathogenic *P. mirabilis* has been implicated as a virulence factor in enhancing the ability of the organisms to infect the urinary tract (Zhao et al., 1999).

The antibiotic susceptibility of *P. myxofaciens* is not known but a feature common to the other three *Proteus* species is an intrinsic resistance to bacitracin and the polymyxins (Potee et al., 1954; Von Graevenitz and Nourbakhsh, 1972; Shimizu et al., 1977). Susceptibility to colistin (colymycin) is rare among *Proteus* species, and if a strain is susceptible to this antibiotic, it is most likely not to be a *Proteus* (Li and Miller, 1970). These cyclic polycationic antibiotics are believed to bind to the charged Kdo-lipid A region of the LPS to cause membrane disruption. Substitution of the phosphate ester bonds of the lipid A with L-arabinoso-4-amine in *Salmonella typhimurium* leads to resistance of the bacteria to polymyxin (Vaara et al., 1981). *Proteus* strains have lipid A that

contains L-arabinoso-4-amine naturally, and this has been suggested as a contributing factor for their natural resistance to these antibiotics (Sidorczyk et al., 1987; Rózalski et al., 1997).

P. mirabilis is the species most frequently isolated from human infections and is generally also the most susceptible to antibiotics, but some very resistant isolates have been reported (Penner et al., 1982). Increasing resistance, particularly to tetracyclines, has been noted since the 1950s (Potee et al., 1954; Chiu and Hoeprich, 1961). The majority of the strains are susceptible to chloramphenicol, but many carry an inducible chloramphenicol acetylase gene (Charles et al., 1985) that could lead to failure in the treatment of brain abscesses (Hawkey, 1997). The occurrence of both nitrofurantoin-sensitive and nitrofurantoin-resistant strains in clinical specimens has been reported.

P. mirabilis is the most susceptible of the three species to the β-lactam group of antibiotics, although resistant strains occur and the necessity of performing antibiotic susceptibility tests is stressed (Barry and Hoeprich, 1973; Shafi and Datta, 1975). *P. mirabilis*, unlike the other two species, does not appear to carry a chromosomal cephalosporinase or an AmpC type penicillinase, and thus many infections can be treated with either cephalosporins or ampicillin (Livermore, 1995). Only rarely is imipenem-resistant *P. mirabilis* isolated, and it has been suggested to be due to altered penicillin-binding proteins (Neuwirth et al., 1995).

Most strains of *P. mirabilis* are susceptible to aminoglycosides, but some strains may acquire resistance factors (R factors) that cause resistance to gentamicin and kanamycin. Amikacin is effective against *P. mirabilis* strains but some have been reported to be resistant to this antibiotic as well as to gentamicin (Briedis and Robson, 1976; Drasar et al., 1976). Resistant *P. mirabilis* can be anticipated to be more frequently encountered in the future, especially in hospital outbreaks, as increased usage has been shown in the past to lead to increased resistance.

Antibiotics generally used to treat infections caused by *P. mirabilis* are trimethoprim or one of the fluoroquinolones such as ciprofloxacin. However, susceptibility tests should be performed for each isolate (Hawkey, 1997).

P. vulgaris is separable into two biogroups, but most of the data on antibiotic susceptibility do not distinguish strains on this basis, and results of antibiotic susceptibility studies in earlier periods did not distinguish between *P. vulgaris* and *P. penneri*. Assessments of the susceptibilities of *P. vulgaris* have been made based on mixtures of strains. However, it is now clear that *P. vulgaris* strains are more resistant than *P. mirabilis* strains. The high levels of *P. vulgaris* resistance to penicillins and cephalosporins is a feature distinguishing it from *P. mirabilis*. Carbenicillin is the only penicillin that has been shown to have significant activity against *P. vulgaris* strains. Most strains, however, are susceptible to aminoglycosides. A small percentage are resistant to gentamicin and kanamycin, but, in most cases, are susceptible to amikacin. Both chloramphenicol-resistant and chloramphenicol-susceptible *P. vulgaris* strains have been reported to be present in clinical specimens, but it is not known what proportion of these would now be classified as *P. penneri*, which are characteristically resistant to this antibiotic (Hickman et al., 1982c).

The high levels of resistance of *P. penneri* strains to chloramphenicol is a definitive criterion in the description of the species (Hickman et al., 1982c). Although increasing numbers of laboratories are identifying *P. penneri* strains, the numbers reported in susceptibility studies are relatively small for a general assessment of the susceptibility of the species. Reports from several laboratories, however, confirm the resistance to chloramphenicol as a species characteristic (Hickman et al., 1982c; Hawkey et al., 1983; Fuksa et al., 1984; Piccolomini et al., 1987). It also appears that the species is more resistant to penicillins than is *P. vulgaris*. In a study of 45 isolates, it was found that ceftizoxime, ceftazidime, moxalactam, and cefoxitin would be effective for treatment of *P. penneri* infections (Fuksa et al., 1984). In another study of 39 isolates, imipenem was found to be the most effective antibiotic against isolates of this species (Piccolomini et al., 1987). Isolates were also found to be susceptible to four aminoglycosides (gentamicin, tobramycin, netilmicin, and amikacin), and their use was recommended for treatment of systemic infections (Fuksa et al., 1984). Isolates of the three species are susceptible to the quinolones, particularly to ciprofloxacin and norfloxacin, but a small number of *P. penneri* isolates show higher levels of resistance (Hawkey and Hawkey, 1984).

P. vulgaris, *P. mirabilis*, and *P. penneri* are important human pathogens causing primary and secondary infections. Of the three species, *P. mirabilis* is most frequently isolated from clinical specimens and is one of the most important pathogens of the urinary tract. Underlying conditions such as diabetes or structural abnormalities of the urinary tract are associated with infections acquired outside the hospital (Grossberg et al., 1962; Wallace and Petersdorf, 1971). Hospital-acquired infections commonly occur in patients with predisposing conditions such as catheterization, urological instrumentation, or surgery of the urinary tract. Infectious strains are transmitted from the intestinal flora of the patient or contracted through transmission from other patients or from a common reservoir (Dutton and Ralston, 1957; Kippax, 1957; Chow et al., 1979). *Proteus* urinary tract infections may give rise to bacteremias that are difficult to treat and often fatal. *Proteus* bacteria may cause infections at other sites of the body if favorable conditions exist. They may cause infections of wounds, burns, and respiratory tracts and have been isolated from the eyes, ears, and the throat. *Proteus* strains may also infect the neonatal umbilical stump, and fatal bacteremias and meningitis may result (Becker, 1962; Librach, 1968; Shortland-Webb, 1968; Burke et al., 1971). *P. mirabilis* has also been isolated from both children and adults with osteomyelitis (Levy and Ingall, 1967; Meyers et al., 1973).

Proteus species have been implicated as causative agents of enteritis and were isolated from 46.2% of infants with diarrhea (Lányi, 1956). However, in light of the fact that approximately one-quarter of the population are intestinal carriers of *Proteus*, it is difficult to assess this finding as convincing evidence of a role for strains of this genus in the etiology of human enteritis. Furthermore, species of the genus *Campylobacter* that are now known to be major causes of human diarrhea were not recognized at the time of the earlier studies.

Recent research on the modification of peptidoglycan (murein) by bacterial enzymes of Gram-positive and Gram-negative bacteria suggest possible involvement of *P. vulgaris* and *P. mirabilis* in the development of arthritic syndromes. It has been demonstrated that one strain of *P. vulgaris* and 17 strains of *P. mirabilis* possess acetyltransferase activity for converting the muramyl residue of peptidoglycan to the 2,6-diacetylmuramyl derivative (Fleck et al., 1971; Gmeiner and Kroll, 1981; Dupont and Clarke, 1991; Clarke and Dupont, 1992). This has the effect of creating resistance of the peptidoglycan to the hydrolytic enzymes in phagocytic cells and serum, resulting in the persistence and circulation in the host of high molecular weight peptidoglycan fragments, which have been implicated in animal models as the inciting agents of rheumatoid arthritis (Chedid et al., 1978; Cro-

martie, 1981; Clarke and Dupont, 1992). It is also noteworthy that antibodies against *Proteus* species have been detected in patients with rheumatoid arthritis (Ebringer et al., 1985). The possibility that organisms of the genus *Proteus* could be involved in the development of rheumatoid arthritis is an interesting concept, but more research is required (Clarke and Dupont, 1992).

Epidemiological typing is performed at present only for *P. vulgaris* and *P. mirabilis*. For reference work, O-serotyping is the method widely accepted as the standard but it is carried out only in a few laboratories that have the large set of typing antisera (Perch, 1948; Kauffmann, 1966; Penner and Hennessy, 1980; Larsson, 1984; Hawkey et al., 1986b). The antisera are not commercially available, and other systems for typing have been introduced and tested with the objective of enabling clinical laboratories to conduct epidemiological studies without the high costs associated with O-serotyping. Among these alternative methods is the Dienes test (described above), which can be readily performed but lacks specificity (Kippax, 1957; Hickman and Farmer, 1976). The incompatibility of two swarms that produce a line of demarcation between them is believed to be due to both the production of and susceptibility to bacteriocins (Senior, 1977a). Typing based on sensitivity to bacteriocins (proticins) has been advocated since 1965 (Cradock-Watson, 1965), and several systems have been developed (Al-Jumaili, 1975; Senior, 1977b; Kusek and Herman, 1980). In a study using the proticine production and proticine sensitivity method, O serotyping, and the Dienes test, a highly discriminating system for identifying *P. mirabilis* and *P. vulgaris* strains, was achieved (Senior and Larsson, 1983). The results also showed that the Dienes test was useful in determining the presence of a common type among a small number of isolates, but not when applied to a large number of isolates for which testing of many combinations is required. In such cases, proticine typing or O serotyping are more appropriate. The greatest discrimination is provided by using both proticine and O serotyping.

Lytic bacteriophage may also be used to differentiate strains of *P. vulgaris* and *P. mirabilis*; several groups of investigators have described typing schemes (Vieu and Capponi, 1965; France and Markham, 1968; Izdebska-Szymona et al., 1971; Schmidt and Jeffries, 1974; Hickman and Farmer, 1976; Bergan, 1978).

A high level of discrimination among *P. mirabilis* strains may also be achieved by testing the strains for growth inhibition in a selected group of chemicals that does not include antibiotics (Kashbur et al., 1974). This method of typing, referred to as resistotyping, was found to compare favorably with serotyping, phage typing, proticine typing, and the Dienes test.

Traditional methods of typing are now being supplanted by new techniques as advances are made in molecular biology. Protein profiles of outer membranes and multilocus enzyme electrophoresis analysis have been used to differentiate strains of *P. vulgaris* and *P. mirabilis* (Kappos et al., 1992). Arbitrarily primed polymerase chain reaction (AP-PCR) and ribotyping have been exploited in a study of *P. mirabilis* isolated in a pediatric hospital (Bingen et al., 1993). With increasing interest in epidemiology, along with expected future simplifications of DNA fingerprinting methods, it is anticipated that more studies will be undertaken to advance our understanding of the epidemiology of these pathogens.

Early studies have shown that *Proteus* strains are widely distributed in nature, occurring in manure, soil, and polluted water, where they are believed to have important roles in the decomposition of organic matter (Wilson and Miles, 1975). They are also present in the intestinal contents of both wild and domesticated animals as well as in the intestines of patients and healthy humans. Incidences of 17–24% for *P. mirabilis* and 3–5% for *P. vulgaris* in healthy humans have been reported (Rustigian and Stuart, 1945; Krikler, 1953). These results were obtained without knowledge that *P. penneri* existed in the genus. Special notice of indole-negative strains was usually not taken, as these would have been considered as atypical indole-negative *P. vulgaris* or atypical ornithine decarboxylase-negative *P. mirabilis* (Müller, 1986a). Thus, their strains identified as *P. vulgaris* may or may not have included *P. penneri* strains. It is noteworthy that in a recent study of bacteria in fecal and urine contaminated bedding in calf-rearing units, *P. vulgaris* and *P. mirabilis*, but no *P. penneri*, strains were isolated (Hawkey et al., 1986b). It seems premature, therefore, to assume that the distribution of *P. penneri* in nature is similar to that of *P. vulgaris*. However, it has been found that *P. penneri* does occur in human feces. Müller (1986a) isolated 13 (0.9%) *P. penneri* from 1422 healthy humans and 20 (1.6%) from 1271 patients. The ecological importance of *Proteus* in the intestine is not known. The hydrolysis of urea is probably minor in comparison to that produced by the large population of urease-producing anaerobes (Sabbaj et al., 1970; Brown et al., 1971), but the oxidative deamination of amino acids may be significant (Drasar and Hill, 1974).

Other human sites of *Proteus* carriage include the urinary tract, the groin area, the vagina, and the prepuce of male infants. The high carriage rate in the intestine has been implicated in the colonization of the groin area (Ehrenkranz et al., 1989) and subsequent development of autoinfections and cross-infections of the urinary tract (Burke et al., 1971). Urine may be considered a habitat of *Proteus* and the source of hospital acquired infections (Hickman and Farmer, 1976; Warren, 1996). The carriage of *Proteus* in the maternal vagina is reported be the source of neonatal infections (Bingen et al., 1993). The carriage of *P. mirabilis* in the prepuce of the infant is believed to be the source of infections in male babies (Glennon et al., 1988).

P. myxofaciens has been isolated only from living and dead gypsy moth larvae (*Porthetria dispar*), but a role in the production of disease in these insects has not been established (Cosenza and Podgwaite, 1966).

Enrichment and Isolation Procedures

Isolation media in the clinical laboratory are formulated to inhibit the swarming of *Proteus* spp. and to select the well-known pathogens such as *Salmonella* and *Shigella*. Such media are suitable for the isolation of *Proteus* spp., but special media for the primary isolation of *Proteus* spp. have been designed (Zarett and Doetsch, 1949; Malinowski, 1966; Xilinas et al., 1975; Hawkey et al., 1986a).

For enrichment of *Proteus* species in fecal samples, tetrathionate or selenite broth have been found to increase the rate of isolation of *P. mirabilis* from 8.2% to 23.6% and *P. vulgaris* from 0% to 2.7% when enrichment preceded plating (Hynes, 1942; Rustigian and Stuart, 1945).

Maintenance Procedures

Proteus strains may be maintained on trypticase soy agar at 4°C with monthly transfers or may be preserved indefinitely by lyophilization. At the Centers for Disease Control and Prevention, cultures in tubes of blood agar base or trypticase soy agar sealed with a cork or rubber stopper remain viable at room temperature for many years without transfer (J.J. Farmer and F.W. Hickman, personal communication).

Differentiation of the genus *Proteus* from other genera

Key characteristics for differentiating the genera *Proteus*, *Providencia*, and *Morganella* are shown in Table BXII.γ.254.

Further Reading

Belas, R. 1992. The swarming phenomenon of *Proteus mirabilis*. ASM News. *58*: 15–22.

Brenner, D.J., J.J. Farmer, III, G.R. Fanning, A.G. Steigerwalt, P. Klykken, H.G. Wathen, F.W. Hickman and W.H. Ewing. 1978. Deoxyribonucleic acid relatedness in species of *Proteus* and *Providencia*. Int. J. Syst. Bacteriol. *28*: 269–282.

Brenner, D.J., F.W. Hickman-Brenner, B. Holmes, P.M. Hawkey, J.L. Penner, P.A. Grimont and C.M. O'Hara. 1995. Replacement of NCTC 4175, the current type strain of *Proteus vulgaris*, with ATCC 29905. Request for an opinion. Int. J. Syst. Bacteriol. *45*: 870–871.

Clarke, A.J. and C. Dupont. 1992. *O*-acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. Can. J. Microbiol. *38*: 85–91.

Kotelko, K. 1986. *Proteus mirabilis*: taxonomic position, peculiarities of growth, components of the cell envelope. Curr. Top. Microbiol. Immunol. *129*: 181–215.

Larsson, P. 1984. Serology of *Proteus mirabilis* and *Proteus vulgaris*. Methods Microbiol. *14*: 187–214.

Mobley, H.L. and R.P. Hausinger. 1989. Microbial ureases: significance, regulation, and molecular characterization. Microbiol. Rev. *53*: 85–108.

TABLE BXII.γ.254. Characteristics differentiating *Proteus*, *Morganella*, and *Providencia*[a,b]

Characteristic	*Proteus*	*Morganella*	*Providencia*
Swarming	+	−	−
H_2S production	+	−	−
Gelatin hydrolysis	+	−	−
Lipase (corn oil)	+	−	−
Utilization of citrate (Simmons)	D	−	+
Ornithine decarboxylase	D	+	−
Acid production from:			
Maltose	D	−	−
D-Mannose	−	+	+
Acid from one or more of the following polyhydric alcohols:			
i-Inositol, D-mannitol, adonitol, D-arabitol, erythritol	−	−	+[c]

[a]For symbols, see standard definitions.

[b]Temperature of reactions, 36° ± 1°C. All reactions for 48 h.

[c]One *Providencia* species (*P. rustigianii*) does not produce acid from polyhydric alcohols.

Mobley, H.L., M.D. Island and R.P. Hausinger. 1995. Molecular biology of microbial ureases. Microbiol. Rev. *59*: 451–480.

Rózalski, A., Z. Sidorczyk and K. Kotelko. 1997. Potential virulence factors of *Proteus bacilli*. Microbiol. Mol. Biol. Rev. *61*: 65–89.

Differentiation of the species of the genus *Proteus*

The key differentiating characteristics for the four species of the genus *Proteus* are shown in Table BXII.γ.255. Additional characteristics of the species are provided in Table BXII.γ.256.

List of species of the genus Proteus

1. **Proteus vulgaris** Hauser 1885, 12[AL] emend. Brenner, Hickman-Brenner, Holmes, Hawkey, Penner, Grimont and O'Hara 1995, 870.
vul.ga′ris. L. adj. *vulgaris* common.

   Morphological characteristics as described for the genus. Other characteristics are listed in Tables BXII.γ.254, BXII.γ.255, and BXII.γ.256. Since the last edition of the *Manual*, indole-negative strains formerly classified as *P. vulgaris* biogroup 1 have been assigned to a new species, *Proteus penneri*. *P. vulgaris* at present includes two biogroups. Biogroup 2 strains are positive for indole, salicin, and esculin and have other reactions characteristic of the vast majority of strains in the species. The present type strain and a very small heterogeneous group belong to biogroup 3, which is indole positive but negative in both the salicin and esculin reactions. Additional species will probably be identified in these biogroups.

   Some strains are hemolytic on blood agar. Generally resistant to penicillins and cephalosporins. Generally more resistant to antibiotics than *P. mirabilis*. Causes infections of the human urinary tract and other sites but not as frequently as *P. mirabilis*. Widely distributed in nature, occurring in human and animal intestines and in contaminated soil and water.

   The mol% G + C of the DNA is: 39.3 ± 1.2 (T_m) (Falkow et al., 1962).

   Type strain: ATCC 29905, DSM 13387.

2. **Proteus mirabilis** Hauser, 1885, 34.[AL]
mi.ra′ bi.lis. L. adj. *mirabilis* wonderful.

   Morphological characteristics are as described for the genus. Other characteristics are listed in Tables BXII.γ.254, BXII.γ.255, and BXII.γ.256. Characteristically indole and maltose negative and ornithine decarboxylase positive. Some strains are hemolytic on blood agar. More frequently isolated from clinical specimens than *P. vulgaris*. Most common site of infection is the human urinary tract. Generally susceptible to penicillins and cephalosporins and more susceptible than *P. vulgaris*.

   The mol% G + C of the DNA is: 39.3 ± 1.45 (T_m) (Falkow et al., 1962).

   Type strain: ATCC 29906.

   GenBank accession number (16S rRNA): AF008582.

3. **Proteus myxofaciens** Cosenza and Podgwaite 1966, 188.[AL]
myx.o.fac′i.ens. Gr. fem. n. *myxa* slime; M.L. masc. n. *faciens* producing; *myxofaciens* slime-producing (bacteria).

   Morphological characteristics as described for the genus. Other characters are listed in Tables BXII.γ.254, BXII.γ.255, and BXII.γ.256. Only one isolate studied in detail. Thin film of growth on solid media. Produces highly viscous slime when cultured in trypticase soy broth. Hemolytic on blood agar. Isolated from living and dead gypsy moth larvae (*Porthetria dispar* L).

   The mol% G + C of the DNA is: not determined.

   Type strain: ATCC 19692.

TABLE BXII.γ.255. Differentiation of the species and biogroups of the genus *Proteus*[a,b]

Characteristic	*P. vulgaris* BG2	*P. vulgaris* BG3	*P. mirabilis*	*P. myxofaciens*[c]	*P. penneri*
Indole production	+	+	−	−	−
Ornithine decarboxylase	−	−	+	−	−
Acid from:					
Maltose	+	+	−	+	+
D-Xylose	+	+	+	−	+
Salicin	+	−	−	−	−
Esculin hydrolysis	+	−	−	−	−
Tyrosine clearing	+	+	+	−	+
Slime producing[d]	−	−	−	+	−

[a]For symbols, see standard definitions.

[b]Temperature of all reactions, except slime production, at 36° ± 1°C.

[c]Reactions based on study at one strain (ATCC 19692).

[d]Slime production at 25°C in trypticase soy broth.

TABLE BXII.γ.256. Other characteristics of species of genus *Proteus*[a,b]

Characteristic	*P. vulgaris*[c]	*P. mirabilis*	*P. myxofaciens*[d]	*P. penneri*[e]
Phenylalanine deaminase	+	+	+	+
Urease	+	+	+	+
NO_3^- reduced to NO_2^-	+	+	+	+
Motility	+	+	+	d
Swarming	+	+	+	d
Gelatin liquefaction (22°C)	+	+	+	d
H_2S production (TSI)	+	+	+ (3–4)[f]	−
Growth in KCN	+	+	+	+
Acid from glucose	+	+	+	+
Methyl red test	+	+		+
Voges–Proskauer test	−	d	+	−
Citrate utilization (Simmons)	d	d	+	d
Tartrate utilization	+	d	+	+
Acetate utilization	d	d	+	−
Lipase activity (corn oil)	d	d	−	d
Deoxyribonuclease (25°C)	d	d	−	d
Oxidase test	−	−	−	−
ONPG hydrolysis	−	−	−	−
Pectate liquefaction	−	−	−	
Malonate utilization	−	−	−	−
L-Lysine	−	−	−	−
L-Arginine	−	−	−	−
L-Ornithine	−	+	−	−
Acid production from:				
Adonitol	−	−	−	−
L-Arabinose	−	−	−	−
D-Arabitol	−	−	−	−
Cellobiose	−	−	−	−
Dulcitol	−	−	−	−
Erythritol	−	−	−	−
Glycerol	d	+	+	+ (7)
i-Inositol	−	−	−	−
Lactose	−	−	−	−
D-Mannitol	−	−	−	−
D-Mannose	−	−	−	−
α-CH_3-glucoside	d	−	+	+ (7)
Raffinose	−	−	−	−
L-Rhamnose	−	−	−	−
D-Sorbitol	−	−	−	−
Sucrose	+	d	−	+
Esculin hydrolysis	d	−	−	−
Mucate	−	−	−	−
ONPG	−	−	−	−

[a]For symbols, see standard definitions.

[b]Biochemical reaction at 36° ± 1°C unless otherwise indicated.

[c]Includes strains of biogroup 2 and biogroup 3 (Brenner et al., 1978).

[d]Reactions based on study of one strain (Brenner et al., 1978).

[e]Reactions as reported by Hickman et al. (1982c).

[f]Number in parentheses indicates number of days for test.

4. **Proteus penneri** Hickman, Steigerwalt, Farmer III and Brenner 1983, 438[VP] (Effective publication: Hickman, Steigerwalt, Farmer III and Brenner 1982c, 1100.)
penn.er'i. M.L. gen. n. *penneri* of Penner; named after the Canadian microbiologist J.L. Penner.

Morphological characteristics are as described for the genus. Other characteristics are listed in Tables BXII.γ.254, BXII.γ.255, and BXII.γ.256. Formerly classified as *P. vulgaris* biogroup 1. Strains are characteristically negative in indole, maltose, salicin, and esculin reactions. Some strains are hemolytic on blood agar. A pathogen of the human urinary tract. Very resistant to chloramphenicol.

The mol% G + C of the DNA is: 38 (T_m).
Type strain: ATCC 33519, CDC 1808-73.

Genus XXX. **Providencia** *Ewing 1962, 96*[AL]

JOHN L. PENNER

Pro.vi.den'ci.a. M.L. fem. n. *Providencia* Providencia, named after the city of Providence, Rhode Island, U.S.A.

Straight rods, 0.6–0.8 × 1.5–2.5 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile by peritrichous flagella. They are **facultatively anaerobic**, chemoorganotrophic, having **both a respiratory and a fermentative type metabolism**. Optimal growth temperature is 37°C. **Oxidatively deaminate phenylalanine and tryptophan**. **Acid is produced from D-mannose**. **All except *Providencia rustigianii* produce acid from one or more of the following polyhydric alcohols**: inositol, D-mannitol, adonitol, D-arabitol, and erythritol. Except for *Providencia heimbachae* all are indole positive. Tartrate (Jordan) is utilized by all but a few strains of *P. heimbachae*. Simmons citrate is utilized by all species except *P. heimbachae* and some strains of *P. rustigianii*. Isolated from diarrhetic stools, urinary tract infections, wounds, burns, bacteremias, and contaminated environmental sources. DNA relatedness tests have shown that *Providencia* are more closely related to *Proteus vulgaris* and *Proteus mirabilis* than to other members of the family *Enterobacteriaceae*. Although 16S RNA sequence data are not available for the *Providencia* species, the close relationship of *Providencia* to *Proteus* indicates that *Providencia*, like *Proteus*, belongs to the *Gammaproteobacteria* within the family *Enterobacteriaceae*.

The mol% G + C of the DNA is: 39–42 (Falkow et al., 1962).

Type species: **Providencia alcalifaciens** (de Salles Gomes 1944) Ewing 1962, 96 (*Eberthella alcalifaciens* de Salles Gomes 1944, 183; *Proteus inconstans* (Ornstein 1921) Shaw and Clarke 1955, 155.)

FURTHER DESCRIPTIVE INFORMATION

Since the last edition of the *Manual*, the genus has been extended to include two new species, *P. rustigianii* and *P. heimbachae*. All *Providencia* species, like those of *Proteus* and *Morganella*, deaminate phenylalanine and other amino acids to produce α-keto and α-hydroxycarboxylic acids (Bernheim et al., 1935; Stumpf and Green, 1944; Singer and Volcani, 1955). The carboxylic acids that actively bind iron have siderophore activity (Drechsel et al., 1993). A probe for the amino acid deaminase (*aad*) of *Proteus mirabilis* does not hybridize with DNA from *Providencia* and *Morganella* strains, indicating unrelatedness of the deaminases of these three genera (Massad et al., 1995). In the clinical laboratory, tests for phenylalanine deaminase are commonly used to identify strains belonging to these three genera (Henriksen, 1950; Thibault and Le Minor, 1957).

Providencia, like *Proteus* and *Morganella*, decomposes tyrosine to produce a clearing on the agar medium in which the insoluble amino acid is incorporated (Sheth and Kurup, 1975) and produces a reddish-brown pigment on nutrient agar containing 5% tryptophan (Polster and Svobodová, 1964) or other aromatic amino acids (Müller et al., 1986). *Providencia* species, with the exception of *P. rustigianii*, differ from *Proteus* and *Morganella* in their ability to produce acid from inositol and straight-chain tetra-, penta-, or hexahydroxy- alcohols, and the species are differentiated based on their reactions on these substrates. *Providencia rettgeri* is the most noted for this property, as most strains of the species are capable of producing acid from several or all of these polyhydric alcohols (Penner et al., 1975). In contrast, *P. rustigianii* strains do not produce acid from any of the polyols. Yellowish orange-centered colonies are known to be produced on deoxycholate citrate agar by *P. alcalifaciens*, *P. rettgeri*, and *P. stuartii* (Cook, 1948; Buttiaux et al., 1954). *P. heimbachae* and *P. rustigianii* have not been examined for this property. The color is apparently caused by the precipitation of ferric hydroxide because of the alkalinity produced by the growth of the bacteria on the medium (Catsaras et al., 1965).

Urease is produced characteristically by strains of *P. rettgeri* and variably by strains of *P. stuartii* (Penner et al., 1976c; Farmer et al., 1977; Mobley et al., 1985). In a hospital outbreak of urinary tract infections, some patients were found to be infected with both urease-positive and urease-negative strains of *P. stuartii* (Penner et al., 1976c). The proportion of the urease-positive strains in *P. stuartii* has been estimated to be as low as 15–16% (Brenner et al., 1978; Penner et al., 1979b) and as high as 24% and 30% (Mobley et al., 1985; Mobley et al., 1986a). The urease enzyme of at least some *P. stuartii* strains was found to be transmissible (Grant et al., 1981) on an 82-kb plasmid (Mobley et al., 1986b). Its molecular weight has been estimated to be 375,000 ± 35,000 (Mobley et al., 1986b). The presence of the plasmid in endemic strains of some hospitals could be the cause of variations among hospitals in the frequencies of isolations of urease-positive *P. stuartii* (Penner et al., 1979b) and the failure to identify the species correctly with commercial identification systems (Cornaglia et al., 1988). A key test for separating *P. stuartii* from *P. rettgeri*, particularly from those aberrant *P. rettgeri* in fermenting D-arabitol or erythritol or both, is the fermentation of trehalose (Fischer et al., 1989). Of the *Providencia* species, only *P. stuartii* strains ferment this compound, and thus the test serves to separate this species from others of the genus.

The production of indole is an important test in the identi-

fication of *Providencia*. All species give positive reactions in this test except *P. heimbachae* (Müller et al., 1986). However, it should be noted that strains of *P. heimbachae* may produce positive reactions after 1 d of incubation when tryptone or peptone media with 0.1% tryptophan are used (Müller, 1986b). To avoid false-positive reactions tryptone media without added tryptophan has been recommended (Müller, 1986b).

Although the strains of *Providencia* are cited as unable to produce acid from lactose, some variants have been reported. An unusual strain of *P. rettgeri* that fermented lactose was reported to be associated with an outbreak of nosocomial urinary tract infection (Traub et al., 1971) and a group of 31 lactose positive isolates of *P. rettgeri* were obtained from hospitalized patients with urinary tract infections (Richard et al., 1974). In the latter study, it was found that the lactose-positive trait was not transferable by conjugation. Five lactose-positive strains were also identified among a collection of 699 *P. stuartii* obtained from urinary tracts of catheterized patients (Mobley et al., 1985). The lactose fermentation was transferable by a 150-kb plasmid (Mobley et al., 1985). In addition to urease and lactose fermentation, acid production from sucrose has also been shown to be plasmid-mediated in *P. stuartii*, indicating that variants in this species are more likely to be the results of plasmid-encoded traits than is generally recognized (Mobley et al., 1985).

Antigenic structure Like other Gram-negative species, *Providencia* possesses the surface arrays of antigenic molecules that may serve as markers for differentiation of the strains. The lipopolysaccharide (O) antigens have been the most widely used for serotyping. The original serotyping scheme for *Providencia* included both *P. alcalifaciens* and *P. stuartii* and recognized 56 O antigens, 28 flagellar (H) antigens, and two capsular (K) antigens (Ewing et al., 1954). The O serotyping scheme has since been separated according to species and extended, so that currently 46 O antigens for *P. alcalifaciens* and 17 O antigens for *P. stuartii* may be identified (Penner et al., 1976b, 1979a, b). The original scheme proposed for serotyping *P. rettgeri* listed 34 O antigens and 26 H antigens (Namioka and Sakazaki, 1958). The O serotype reference strains (serostrains) of this scheme were included in a revised and extended scheme that currently recognizes 93 O specificities (Penner et al., 1974, 1976a). In the schemes for O serotyping the three *Providencia* species by Penner and colleagues, the passive (indirect) hemagglutination technique was used instead of the slide agglutination technique to identify the O antigens because it provided greater specificity, sensitivity, and reproducibility of results. This is due to the 228-kDa lipoglycoprotein receptor on the erythrocyte membrane that binds lipopolysaccharide specifically and does not bind proteins or polysaccharides (Springer et al., 1973). Thus, the possible participation in passive hemagglutination of flagellar, fimbrial, or capsular antigens is largely excluded from the antigen-antibody reactions. The O serotyping schemes have been applied in a number of epidemiological investigations and in the study that advocated the reassignment of the *P. rettgeri* biogroup 5 strains to *P. stuartii* (Penner et al., 1975; Penner and Hennessy, 1977). At present, serotyping schemes for *P. heimbachae* and *P. rustigianii* have not been developed. However, three strains of *P. rustigianii*, NCTC 9259, NCTC 9195, and NCTC 9235, originally classified as *P. alcalifaciens* biogroup 3 (Ewing et al., 1972), were included in the present serotyping scheme for *P. alcalifaciens* as the reference strains for serotypes O:10, O:14, and O:54, respectively (Penner et al., 1976b). Strains of these serotypes have not been identified in any epidemiological study on *Providencia* (Penner et al., 1979a).

Although it is highly probable that strains of the same O serotype from the same epidemiological setting are the same and thus important for investigating outbreaks, strains from different sources that have the same O antigens may not be the same. In such cases, a second method of discriminating between isolates is necessary. Using plasmid content to differentiate strains of *P. rettgeri*, it was shown that environmental strains of serotype O:58 were not identical (Hawkey et al., 1986b).

Although flagellar (H) antigens have been defined for some of the serotype reference strains of *P. alcalifaciens* and *P. stuartii* (Ewing et al., 1954) and for some *P. rettgeri* (Namioka and Sakazaki, 1958; Penner and Hinton, 1973), practical use of H typing has been limited to a few isolates examined in the earlier studies. Capsular antigens have also not been used as biomarkers for differentiating strains of this genus. One capsular antigen of *P. rettgeri* has been examined and found to be unlike the typical K antigens of *Enterobacteriaceae* (Namioka and Sakazaki, 1959). It is different in that it retains antibody-binding capacity after heat treatment at 100°C for 1 h and the antigenic specificity is restricted to particular O groups. The authors proposed the designation of C to distinguish this capsular antigen from the *Escherichia coli* L and B antigens and suggested that there are numerous serological types of C antigens.

Numerical analysis of electrophoretic protein patterns Serotyping based on the O antigens has its drawbacks for routine work in the clinical laboratory, because large numbers of commercially unavailable antisera are required. A promising alternative to serotyping is the application of high resolution polyacrylamide gel electrophoresis (PAGE) of proteins combined with computerized analysis of patterns This approach has been used successfully to determine the PAGE types among strains of *P. alcalifaciens*, *P. rettgeri*, *P. stuartii*, and *P. rustigianii* (Costas et al., 1987, 1989, 1990; Holmes et al., 1988). No strains tested by this procedure were serotyped, and although the two methods have not been correlated, the results obtained by determination of the PAGE types suggest the method is as discriminating as serotyping (Costas et al., 1990). Moreover, computerized analysis of electrophoretic protein patterns correlate well with DNA relatedness studies.

Other methods of differentiating strains of *Providencia* species Results have been shown that rDNA fingerprinting also permits discrimination among strains of *P. stuartii* and their differentiation from other species of *Providencia* and *Proteus* (Owen et al., 1988).

Another alternative to serotyping is to discriminate among strains based on their sensitivity and resistance to bacteriocins. An extensive scheme employing 12 bacteriocins (provicines) to discriminate among isolates of *P. alcalifaciens* and *P. stuartii* demonstrated that 37 sensitivity patterns occurred among 300 isolates of these two species, most of which were *P. stuartii* (Al-Jumaili and Fenwick, 1978). One provicine sensitivity pattern accounted for 32 (~10%) of the isolates, seven patterns accounted for ~40%, and the remaining 29 patterns accounted for 40%. The number of patterns and the distribution of the isolates among the different patterns indicated that this system of typing would be applicable in epidemiological investigations of *Providencia* infections caused by these two species.

Fimbriae and hemagglutination In *Providencia*, as in other *Enterobacteriaceae*, fimbriae of different types are present but their distribution is more complex than in most other genera of the family (Old and Adegbola, 1982). The presence of six different types is demonstrable by electron microscopy. The *Providencia* fimbriae that have been examined in most detail are those of *P. stuartii* (Old and Scott, 1981; Old and Adegbola, 1982; Mobley et al., 1988). Three fimbrial types have been examined for hemagglutinating and adhesive properties. Cells expressing mannose-sensitive (MS) type 2 fimbriae agglutinate guinea pig erythrocytes in the absence but not in the presence of mannose. Mannose-resistant, *Klebsiella*-like (MR/K) type 3 fimbriae agglutinate only tannic acid-treated cells, and mannose-resistant, *Proteus*-like (MR/P) type 4 fimbriae agglutinate both tannic acid-treated and untreated erythrocytes. Evidence suggests that the MR/K fimbriae of this species enable binding to catheter material, an important factor in contributing to its long-term persistence in the urinary tract of infected patients with indwelling catheters (Mobley et al., 1988).

Deoxyribonucleic acid relatedness of *Providencia* species *Providencia* are most closely related to *Proteus*, but both genera are only distantly related to *E. coli* and to other species in the family *Enterobacteriaceae* (Coetzee, 1972; Brenner et al., 1978; Brenner, 1984a). In the biochemical characterization of *P. alcalifaciens* four biogroups were recognized (Ewing et al., 1972). DNA relatedness tests showed that biogroups 1 and 2 are a homogeneous group, but biogroup 3 strains were found to be only 44–49% related to biogroups 1 and 2 and 26–33% related to *P. stuartii* (Brenner et al., 1978; Hickman-Brenner et al., 1983b). The biogroup 3 strains were transferred from *P. alcalifaciens* and assigned to a new species, *P. rustigianii* (Hickman-Brenner et al., 1983b). Another group of strains isolated mostly from feces of several species of penguins resembled most closely the biogroup 3 strains and were placed in another newly described species, *P. friedericiana* (Müller, 1983). In a subsequent collaborative study between Müller and the group at the Centers for Disease Control and Prevention, DNA hybridization testing showed that strains of *P. friedericiana* are very closely related to *P. rustigianii* and it was concluded that the two groups should be included in the same species (Hickman-Brenner et al., 1986). *P. rustigianii* was described first and the species name was validated before *P. friedericiana* and therefore *P. rustigianii* has priority and is the senior of the two subjective synonyms (Hickman-Brenner et al., 1986).

A biogroup of strains from penguins that were initially included in *P. friedericiana* and one strain listed under the CDC Enteric Group 78 (strain CDC 1519-73) were found to constitute a separate hybridization group and have been assigned to *P. heimbachae*, a newly described species (Müller et al., 1986).

The *P. alcalifaciens* biogroup 4 strains, originally reported to be inositol-negative were found on reexamination to be inositol-positive, a characteristic reaction of *P. stuartii*. They are also highly related to *P. stuartii* and have low levels of relatedness to *P. alcalifaciens*, *P. rettgeri*, and to *Proteus*, and have therefore been transferred to *P. stuartii* (Brenner et al., 1978).

In the earlier classification of the *Proteus-Providencia* group, *P. rettgeri* was included in the genus *Proteus* (Ewing and Davis, 1972b). In a biochemical characterization of this species, five biogroups were recognized (Penner et al., 1975). The biogroup 5 strains are urease-positive, a characteristic reaction of strains in the genus *Proteus*, but in other respects they resemble urease-negative *P. stuartii* (Penner et al., 1975, 1976c; Penner and Hennessy, 1977). On the basis of DNA relatedness tests they were found to be inseparable from *P. stuartii* and to be only 30–35% related to typical *Proteus rettgeri* biogroups 1, 2, and 3 and were therefore transferred to *P. stuartii* (Brenner et al., 1978). DNA of the three strains of *P. rettgeri* biogroup 4 (salicin and rhamnose negative) and other strains that have reactions atypical of *P. rettgeri* biogroups 1, 2, and 3 have not been examined for relatedness.

Antimicrobial susceptibilities New insights into species differences in antimicrobial susceptibilities have been noted since the revisions in the classification of genus *Providencia* in 1978 (Brenner et al., 1978). Studies using earlier classifications included urease-positive *P. stuartii* in collections identified as *Proteus rettgeri* in examining strains for antimicrobial susceptibility. Since the general adoption of the revised classification, it has become evident that, with the exception of a few unusual strains, *P. stuartii* is the most resistant species in the genus *Providencia* and one of the most resistant species in the family *Enterobacteriaceae*. *P. alcalifaciens* is the least resistant, being susceptible to cephalosporins, aminoglycosides, and quinolones, whereas *P. rettgeri* is intermediate to *P. alcalifaciens* and *P. stuartii*. Most strains of *P. rettgeri* are susceptible to gentamicin, tobramycin, and amikacin but are markedly resistant to quinolones. Most strains of *P. stuartii* are resistant to gentamicin, tobramycin, and the quinolones (including ciprofloxacin) but are susceptible to amikacin (Penner and Preston, 1980; Penner et al., 1982; Hawkey and Hawkey, 1984). Strains of *P. stuartii* that are also resistant to carbenicillin (due to a 47-kb plasmid) have also been identified (Hawkey et al., 1985). Some of the newer β-lactams, however, may be useful alternatives to amikacin in the treatment of serious *P. stuartii* infections (Hawkey, 1984). Only 11 strains of *P. rustigianii* and 13 of *P. heimbachae* have been examined for antimicrobial susceptibilities. They generally resemble *P. alcalifaciens* in their susceptibility to cephalosporins and aminoglycosides, but a small number differ in resistance to particular antibiotics (Hickman-Brenner et al., 1983b; Müller et al., 1986). 73% of the *P. rustigianii* are resistant to colistin and 100% and 77% of the *P. heimbachae* are resistant to tetracycline and cephalothin, respectively. However, it should not be concluded that these are characteristic species differences because of the small numbers of isolates that were available for study.

Pathogenicity *P. alcalifaciens* has been isolated from human feces, particularly from those of the pediatric population ever since the organism was first described, but whether this species is an etiological agent of human gastroenteritis is still a matter of debate and speculation. Evidence in support of a pathogenic role is the finding in several studies that the organisms are more frequently isolated from patients with diarrhea than from normal individuals (Prakash et al., 1966; Bhat et al.,1971a, b; Albert et al., 1995a, 1998; Guth and Perrella, 1996). Serotyping has shown that isolates of serotype O:3 are more frequently associated with pediatric diarrheas than are other serotypes, and this serotype is not frequently found in healthy children (Carpenter, 1964; Bhat et al., 1971a, b; Penner et al., 1979a). Other serotypes identified less frequently in human cases of diarrhea are O:12, O:13, and O:22 (Stenzel, 1961; Bhat et al., 1971a). In the earlier studies, examinations were not made for the more recently recognized pathogens such as rotaviruses, campylobacteria, pathogenic *E. coli*, *Yersinia enterocolitica*, and intestinal parasites, and therefore the importance of fecal isolates of *P. alcalifaciens* has remained unclear. If the organisms of this species are not causal agents, it

may be that they merely flourish in the intestinal environment created by known diarrhea-causing agents. However, in an investigation of traveler's diarrhea it was found that 10% of returning travelers had stools positive for *P. alcalifaciens*, whereas only 1.3% of stools from a control population with diarrhea were positive (Haynes and Hawkey, 1989). No well-known pathogens were found in any of the samples that were positive for *P. alcalifaciens*, and the authors cited this observation in support of a role for this species in producing human diarrhea. Moreover, strains of this species isolated from diarrheal stools have been shown to be invasive for HEp-2 cells, to be able to produce diarrhea in adult rabbits with removable ileal ties (RITARD model), and to invade the rabbit intestinal mucosa (Albert et al., 1995a, 1998). In addition, some patients infected only with *P. alcalifaciens* experienced manifestations of invasive diarrhea (Albert et al., 1998). In another study, 50% of the *P. alcalifaciens* strains isolated in pure culture or from stool specimens in which no other enteropathogen was identified, were found to be invasive for HeLa cells (Guth and Perrella, 1996). Most of the invasive strains were isolated from diarrheal stools but the invasive characteristic was not present in all *P. alcalifaciens* strains that were isolated from patients with diarrhea. The production of cytotoxins or enterotoxins could not be detected in the invasive *P. alcalifaciens* isolates and their invasiveness could therefore not be attributed to these well-known virulence factors (Albert et al., 1995a, 1998; Guth and Perrella, 1996). It should be noted that an *E. coli* strain that can colonize the small bowel mucosa is unable to produce any of the well-known toxins, but can cause fluid accumulation and diarrhea in the rabbit model (Wanke and Guerrant, 1987). The mechanisms for the production by the *E. coli* strain of the fluid and diarrhea are unknown but the possibility of similar, yet unknown mechanisms, should be considered before discounting an etiological role in the enteropathogenicity of at least some strains of *P. alcalifaciens*. Although evidence is accumulating in support of an etiological role, more direct studies are necessary to provide convincing evidence for the inclusion of this species as a true member of the human enteropathogens.

The urinary tract of the compromised or catheterized patient is the most common site of *P. rettgeri* and *P. stuartii* infections. The rise in importance of these two species is associated with their tendency to cause nosocomial infections and with their marked resistance to numerous antibiotics (Wenzel et al., 1976; Whiteley et al., 1977; Kopf and Freitag, 1979; Penner et al., 1979b, 1981; Kocka et al., 1980; McHale et al., 1981; Warren et al., 1982; Hollick et al., 1984; Warren, 1986). Of the two species, *P. stuartii* is significantly more pathogenic and more resistant (Hawkey, 1984). Earlier studies on *P. rettgeri* often included urease-positive *P. stuartii* strains, thus occluding the significant differences between the two species (Lindsey et al., 1976; Penner et al., 1979b). Since the revision in classification, *P. stuartii* have been the subject of numerous studies to determine the basis of their pathogenicity. It has been shown that the majority of the *P. stuartii* isolated from urinary tract infections adhere to uroepithelial cells *in vitro*, suggesting that this property may account for the persistence of the bacteria in the urinary tract (Mobley et al., 1986a). Further studies have yielded evidence that strains bearing the MR/K fimbriae (mannose-resistant *Klebsiella*-like hemagglutinins) have the exceptional ability of binding to catheters, a characteristic that has been hypothesized to enhance their resistance to antimicrobials and to increase the duration of infection in patients with urinary tract catheters (Mobley et al., 1986a). *P. stuartii* can cause serious infections in surgical and burn wounds and often give rise to fatal septicemias (Curreri et al., 1973; Milstoc and Steinberg, 1973; Wenzel et al., 1976).

In elderly catheterized patients who often have high levels of urinary indoxyl sulfate and have colonization of their urinary tract with *P. stuartii*, the urinary catheter bag develops an intense purple color. This purple urine bag syndrome has been determined to be due to the presence in the organisms of indoxyl sulfatase activity, which produces indigo through the decomposition of the urinary indoxyl sulfate (Dealler et al., 1988).

Although *P. stuartii* has been isolated from human feces, there is no evidence that the species causes infections of the human gastrointestinal tract, but in an investigation of neonatal diarrhea in calves (calf scours), evidence was obtained that suggested an etiological role for *P. stuartii* (Waldhalm et al., 1969) or a contributing role by acting synergistically with a neonatal calf diarrhea virus in the production of such diarrheas (Waldhalm et al., 1974b).

P. rustigianii strains have been isolated from human feces, but only infrequently. In a collection of 891 *Providencia* isolates made by Ewing et al. (1972), only 11 (<2%) were identified as *P. rustigianii* and three of these were reported to have been isolated from stool cultures (Hickman-Brenner et al., 1983b). Of the 19 isolates of this species studied by Costas et al. (1987), 10 were also from human feces (Costas et al., 1987). These studies indicate that bacteria of this species can colonize the human intestinal tract, but the clinical significance of this is not known. Strains of this species were also isolated from penguin feces by Müller (1983), but the significance of the presence of these organisms in the intestine to the health of the animals has not been investigated.

All 13 isolates of *P. heimbachae* that have been studied are from healthy penguins except one isolate that was obtained from an aborted bovine fetus (Müller et al., 1986). The medical and veterinary importance of these organisms remains unknown.

Habitats Early studies to determine the habitats of the *Providencia* were carried out without the advantage of the recently revised classifications, and the prevailing view was that these organisms are widespread in nature, occurring in the intestines of various animals and polluted environmental sources (Müller, 1972). Uncertainty remains on the significance of their presence in the human intestine and only recently have systematic studies been reported to address this issue. From examinations of 1108 stool cultures of pediatric patients 56 isolates of *P. alcalifaciens* were found, and in the same period of time 25 isolates were recovered in routine from three adult hospitals (Penner et al., 1979a). Clearly organisms of this species can be found in the human intestine without special enrichment techniques, but their association with the production of diarrhea remains to be demonstrated.

It has been suspected that *P. stuartii* may also be found in the human intestine, but it is rarely isolated in the clinical laboratory from stool samples. As shown in two studies using special techniques such as pre-enrichment or selective differential media, the organisms of this species occur in the human intestine more frequently than previously believed and may be an important and previously underestimated nosocomial reservoir (Hawkey et al., 1982a, b; Stickler et al., 1985). The major source of the organisms in the hospital setting was found to be the colonized skin of the groin area of patients that were fecal carriers of *P. stuartii* (Stickler et al., 1985). *P. stuartii* was not isolated from raw

sewage and sewage contaminated water samples (Stickler et al., 1985). In a survey of the *Proteus*, *Providencia*, and *Morganella* in the environment of calf rearing units, 127 isolates were recovered of which 50 were *Providencia* (Hawkey et al., 1986b). One was classified as *P. alcalifaciens*, 35 as *P. rettgeri*, and 14 as *P. stuartii*, and it was suggested that a potential cause of colonization of the human intestine by these organisms is the consumption of meat (Hawkey et al., 1986b).

P. rettgeri was first isolated by Rettger from poultry (Hadley et al., 1918). Its occurrence in the intestines of reptiles, frogs, and a duck, and its presence in natural waters have been reported (Müller, 1972; Penner and Hennessy, 1979a). Of 112 isolates collected from the clinical laboratory of a large hospital, 106 were from urine specimens and six were from stool samples (Penner and Hennessy, 1979a). In a study of the environment of calf rearing units in England, *P. rettgeri* was found to be second to *Proteus vulgaris* as the most frequently occurring species (Hawkey et al., 1986b). It thus appears that the species is widely distributed in nature, but is not a common resident of the human intestinal tract.

Studies to determine the habitats of *P. rustigianii* and *P. heimbachae* have not been conducted. In the first description of *P. rustigianii* only 11 isolates were available. Three of these were isolated from stools, but the sources of the other eight isolates are unknown (Hickman-Brenner et al., 1983b). Another group of *P. rustigianii* isolates (originally classified as *P. friedericiana*) were all isolated from feces of different species of penguins (Müller, 1983). Strains of *P. heimbachae* have been isolated only from fecal specimens of penguins and from one aborted bovine fetus (Müller et al., 1986).

Enrichment and Isolation Procedures

Media used in the clinical laboratory for isolation of *Enterobacteriaceae* may be used to isolate *Providencia*. Tetrathionate or selenite broths may be used for enrichment.

A selective differential medium for the detection of *P. alcalifaciens* has been developed by Senior (1997). After enrichment of fecal samples in tetrathionate broth, the broth cultures are plated on a solid agar medium that contains phenol red dye and three sugars (xylose, mannitol, and galactose) that *P. alcalifaciens* does not ferment. Since other enteric bacteria that are present in the tetrathionate broth ferment one or more of these sugars, they produce lemon-yellow (acid-forming) colonies. Because strains of *P. alcalifaciens* do not ferment sugars in this medium, the strains produce red (alkaline) colonies.

Enrichment in heart infusion broth (2.5%) followed by plating on MacConkey agar containing 5 mg/l gentamicin sulfate has been found useful for the isolation of *P. stuartii* (Hawkey et al., 1982b). A selective differential medium for *P. stuartii* contains colistin (as the selective agent) and inositol in a nutrient agar base with bromothymol blue (as the acid-alkali indicator). After overnight incubation at 37°C, *P. stuartii* produces yellow inositol-fermenting colonies, *Proteus mirabilis* produces green-centered colonies, and *Morganella morganii* produces blue colonies (Stickler et al., 1985).

Maintenance Procedures

Providencia strains may be maintained on trypticase soy agar at 4°C with monthly transfers or may be preserved indefinitely by lyophilization. The Enteric Section, Centers for Disease Control and Prevention, stores cultures at room temperature in tubes of blood agar base or trypticase soy agar. These tubes are sealed with a cork or rubber stopper, and the cultures have remained viable for many years without transfer (J.J. Farmer III and F. Hickman-Brenner, personal communication).

Differentiation of the genus *Providencia* from other genera

Characteristics and biochemical reactions for differentiating *Providencia* from the closely related genera, *Proteus* and *Morganella*, are described in Table BXII.γ.254 in the chapter on the genus *Proteus*. An important distinguishing characteristic of the *Providencia* (except *P. rustigianii*) is the ability to produce acid from one or more polyhydric alcohols. Biochemical reactions for differentiating *Providencia* from other species in the family *Enterobacteriaceae* are provided in Tables BXII.γ.193, BXII.γ.194, BXII.γ.195, and BXII.γ.196 in the chapter on *Enterobacteriaceae*.

Taxonomic Comments

Since the last edition of the *Manual*, two new species have been included in the genus *Providencia*. Strains formerly classified as *P. alcalifaciens* biogroup 3 (Ewing et al., 1972) were found to constitute a separate species based on DNA relatedness tests and were assigned the epithet *rustigianii* in honor of Robert Rustigian who is noted for his early work on the genus *Proteus* (Hickman-Brenner et al., 1983b). A group of well-defined strains isolated from fecal samples obtained from penguins were found to represent a new species of the genus, and the epithet *friedericiana* was proposed to recognize the technical contributions of Friederike Heimbach (Müller, 1983). Both of these species gained standing in nomenclature but studies of strains exchanged between the two laboratories led to the finding that the species were the same. Since *P. rustigianii* was validated earlier it has priority and *P. friedericiana* is therefore a (junior) subjective synonym of *P. rustigianii* (Hickman-Brenner et al., 1983b). A biogroup of *P. friedericiana* and a strain (CDC 1519-73) of the "Enteric Group 78" were found in DNA relatedness tests to constitute a separate species that has been assigned the epithet *heimbachae* in honor of Friederike Heimbach, who isolated the original 12 strains of this species (Müller et al., 1986).

Further Reading

Albert, M.J., M. Ansaruzzaman, N.A. Bhuiyan, P.K. Neogi and A.S. Faruque. 1995. Characteristics of invasion of HEp-2 cells by *Providencia alcalifaciens*. J. Med. Microbiol. *42*: 186–190.

Albert, M.J., A.S. Faruque and D. Mahalanabis. 1998. Association of *Providencia alcalifaciens* with diarrhea in children. J. Clin. Microbiol. *36*: 1433–1435.

Brenner, D.J., J.J. Farmer, III, G.R. Fanning, A.G. Steigerwalt, P. Klykken, H.G. Wathen, F.W. Hickman and W.H. Ewing. 1978. Deoxyribonucleic acid relatedness in species of *Proteus* and *Providencia*. Int. J. Syst. Bacteriol. *28*: 269–282.

Hawkey, P.M. 1984. *Providencia stuartii*: a review of a multiply antibiotic-resistant bacterium. J. Antimicrob. Chemother. *13*: 209–226.

Hickman-Brenner, F.W., J.J. Farmer III, A.G. Steigerwalt and D.J. Brenner. 1983. *Providencia rustigianii*: a new species in the family *Enterobacteriaceae* formerly known as *Providencia alcalifaciens* biogroup 3. J. Clin. Microbiol. *17*: 1057–1060.

Müller, H.E., C.M. O'Hara, G.R. Fanning, F.W. Hickman-Brenner, J.M. Swenson and D.J. Brenner. 1986. *Providencia heimbachae*, a new species of *Enterobacteriaceae* isolated from animals. Int. J. Syst. Bacteriol. *36*: 252–256.

Warren, J.W. 1986. *Providencia stuartii*: a common cause of antibiotic-resistant bacteriuria in patients with long-term indwelling catheters. Rev. Infect. Dis. *8*: 61–67.

List of species of the genus Providencia

1. **Providencia alcalifaciens** (de Salles Gomes 1944) Ewing 1962, 96[AL] (*Eberthella alcalifaciens* de Salles Gomes 1944, 183; *Proteus inconstans* (Ornstein 1921) Shaw and Clarke 1955, 155.)
al.cal.i.fac'i.ens. Fr. n. *alcali* alkali; L. v. *facere* to do, make; L. part. adj. *faciens* making; M.L. part. adj. *alcalifaciens* alkali-producing.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.257 and BXII.γ.258. Urease negative. Most strains are susceptible to penicillins, cephalosporins, and aminoglycosides. Organisms are generally isolated from diarrheic stools but also from some normal stools. The species is suspected to be a causal agent of infant diarrhea and has been implicated as a cause of traveler's diarrhea but firm evidence for an etiological role in the production of diarrhea has not been established.

   The mol% G + C of the DNA is: 43 (T_m) (Owen et al., 1987).

   Type strain: ATCC 9886, DSM 30120.

2. **Providencia heimbachae** Müller, O'Hara, Fanning, Hickman-Brenner, Swenson and Brenner 1986, 255.[VP]
heim.bach'ae. M.L. gen. fem. n. *heimbachae* of Heimbach, named to honor Friederike Heimbach who isolated the 12 original strains of the species.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.257 and BXII.γ.258. It can be differentiated from other *Providencia* species by its negative reactions for Simmons citrate, growth in the presence of KCN, acid production from trehalose, urease, and indole production, and by positive tests for acid production from adonitol, D-arabitol, D-galactose, and L-rhamnose. Generally susceptible to penicillins, cephalosporins, and aminoglycosides, but most strains are resistant to cephalothin and tetracycline. Only 13 strains have been available for study. All were isolated from healthy penguins except for one that was isolated from an aborted bovine fetus. The medical and veterinary significance of these organisms is unknown.

   The mol% G + C of the DNA is: 39.6 (T_m) (Owen et al., 1987).

   Type strain: ATCC 35613, DSM 3591.

3. **Providencia rettgeri** (Hadley, Elkins and Caldwell 1918) Brenner, Farmer, Steigerwalt, Klykken, Wathen, Hickman and Ewing 1978, 269[AL] (*Bacterium rettgeri* Hadley, Elkins and Caldwell 1918, 180; *Proteus rettgeri* (Hadley, Elkins and Caldwell 1918) Rustigian and Stuart 1943, 242.)
rett'ge.ri. M.L. gen. n. *rettgeri* of Rettger, named after L.F. Rettger, the American bacteriologist who first isolated the organism in 1904.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.257 and BXII.γ.258. Virtually all strains hydrolyze urea and are distinguished from the other *Providencia* by their ability to produce acid from a variety of polyhydric alcohols. Many strains are resistant to the penicillins and cephalosporins, but the strains are generally more susceptible to aminoglycosides than are *P. stuartii* strains. Generally isolated from urine specimens of hospitalized and catheterized patients and less frequently from other sites. May cause nosocomial infections. Rarely isolated from stool specimens.

   The mol% G + C of the DNA is: 40.5 (T_m) (Owen et al., 1987).

   Type strain: ATCC 29944, DSM 4542.

4. **Providencia rustigianii** Hickman-Brenner, Farmer, Steigerwalt and Brenner 1983a, 673[VP] (Effective publication: Hickman-Brenner, Farmer, Steigerwalt and Brenner 1983b, 1060.)
rus.tig.i.an'i.i. M.L. gen. n. *rustigianii* of Rustigian, named in honor of Robert Rustigian who did early studies on the *Proteus* group.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.257 and BXII.γ.258. Strains are differentiated from other *Providencia* species by their production of acid from D-galactose but not from adonitol, *i*-inositol, and trehalose. Only 11 strains have been examined for antibiotic susceptibly. Most are susceptible to cephalosporins and synthetic penicillins but resistant to natural penicillin and colistin. They may colonize the human intestinal tract but the medical significance of this is not known.

   The mol% G + C of the DNA is: 41.8 (T_m) (Owen et al., 1987).

   Type strain: ATCC 33673, DSM 4541.

5. **Providencia stuartii** (Buttiaux, Osteux, Fresnoy and Mor-

TABLE BXII.γ.257. Differential characteristics of the genus *Providencia*

Characteristic[a]	1. *P. alcalifaciens*	2. *P. heimbachae*	3. *P. rettgeri*	4. *P. rustigianii*	5. *P. stuartii*
Indole production	+	−	+	+	+
Citrate (Simmons)	+	−	+	d	+
Urea	−	−	+	−	d
Motility	+	d	+	d	d
Growth in KCN	+	−	+	+	+
Gas from D-Glucose	d	−	−	d[b]	−
Acid production from:					
Adonitol	+	+	+	−	−
D-Arabitol	−	+	+	−	−
D-Galactose	−	+	+	+	+
i-Inositol	−	d	+	−	+
D-Mannitol	−	−	+	−	−
L-Rhamnose	−	+	d	−	−
Trehalose	−	−	−	−	+

[a]All tests were performed at 36° ± 1°C.

[b]Minimal gas production.

TABLE BXII.γ.258. Other characteristics of the species of the genus *Providencia*

Characteristic[a]	1. *P. alcalifaciens*[b]	2. *P. heimbachae*[c]	3. *P. rettgeri*[b]	4. *P. rustigianii*[d]	5. *P. stuartii*[b]
Methyl red	+	d	+	d	+
Voges–Proskauer	−	−	−	−	−
H_2S on TSI	−	−	−	−	−
Phenylalanine	+	+	+	+	+
L-Lysine (Møller's)	−	−	−	−	−
L-Arginine (Møller's)	−	−	−	−	−
L-Ornithine (Møller's)	−	−	−	−	−
Gelatin (22°C)	−	−	−	−	−
Malonate	−	−	−	−	−
D-Glucose (acid production)	+	+	+	+	+
D-Glucose (gas production)	d	d (7)[e]	d	d	−
Acid from:					
L-Arabinose	−	−	−	−	−
Dulcitol	−	−	−	−	−
Erythritol	−	−	d	−	−
Glycerol	d	d (7)	d	−	d
Lactose	−	−	−	−	−
Maltose	−	+ (7)	−	−	−
D-Mannose	+	+	+	+	+
Melibiose	−	−	−	−	−
α-CH_3-glucoside	−	−	−	−	−
Raffinose	−	−	−	−	−
Salicin	−	−	d	−	−
D-Sorbitol	−	−	−	−	−
Sucrose	d	−	d	+ (7)	d
D-Xylose	−	d (7)	d	−	−
Esculin hydrolysis	−	−	d	−	−
Acid from mucate	−	−	−	−	−
Tartrate (Jordan's)	+	d	+	+	+
Acetate utilization	d	−	d	−	d
Lipase (corn oil)	−	−	−	−	−
DNase 25°C	−	−	−	−	−
$NO_3 \rightarrow NO_2$	+	+	+	+	+
Oxidase production	−	−	−	−	−
ONPG production	−	−	−	−	−
Tyrosine clearing	+	+	+	+	+

[a]Biochemistry reactions at 36° ± 1°C unless otherwise stated.

[b]Data from Brenner et al. (1978).

[c]Data from Müller et al. (1986).

[d]Data from Hickman-Brenner et al. (1983b).

[e]Numbers in parentheses indicate number of days for test.

iamez 1954) Ewing 1962, 96[AL] (*Proteus stuartii* Buttiaux, Osteux, Fresnoy and Moriamez 1954, 385; *Proteus inconstans* (Ornstein 1921) Shaw and Clarke 1955, 155.)
stu.ar′ti.i. M.L. gen. n. *stuartii* of Stuart, named after C.A. Stuart, American bacteriologist who did much of the early work on *Providencia*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.257 and BXII.γ.258. Highly resistant to antibiotics. Some strains are among the most resistant of the *Enterobacteriaceae*. Strains may be urease positive or urease negative. Isolated most often from urine specimens of hospitalized and catheterized patients. Some strains have the ability to adhere to catheters and thus enhance resistance to treatment and increase the duration of the infection. Less frequently isolated from wounds, burns, and bacteremias. They occur in small numbers in the intestines of some individuals and are rarely isolated routinely from stool cultures in the clinical laboratory but serve as the source for the colonization of the skin of the groin area, which may lead to autoinfections and cross-infections.

The mol% G + C of the DNA is: 40.7 (T_m) (Owen et al., 1987).

Type strain: ATCC 29914, DSM 4539.

Genus XXXI. **Rahnella** *Izard, Gavini, Trinel and Leclerc 1981c, 382[VP] (Effective publication: Izard, Gavini, Trinel and Leclerc 1979, 174)*

PETER KÄMPFER

Rah′nel.la. M.L. dim. ending *-ella*; M.L. fem. n. *Rahnella* named after Otto Rahn, the German-American microbiologist and who proposed the name *Enterobacteriaceae* in 1937.

Straight rods 0.5–0.7 × 2–3 μm, conforming to the general definition of the family *Enterobacteriaceae*. **Gram negative, motile by peritrichous flagella when grown at 25°C.** Facultatively anaerobic. Chemoorganotrophic. **Psychrotolerant**, growing at 4°C. D-Glucose is fermented with the production of acid and, for the majority of strains, gas. Nitrate is reduced to nitrite. Oxidase neg-

ative, catalase positive. **Negative for lysine and ornithine decarboxylases and for arginine dihydrolase. Most strains are (weakly) positive for phenylalanine deaminase (after 48 h), methyl red, and Voges–Proskauer reaction. Acids are produced from various carbohydrates, including L-arabinose, cellobiose, lactose, maltose, mannose, L-rhamnose, raffinose, D-xylose, and salicin.** Sequence analyses of the 16S rDNA of four *Rahnella* strains clearly placed the genus within the *Gammaproteobacteria* in the family *Enterobacteriaceae*. Highest 16S rDNA sequence similarity to *Yersinia enterocolitica* ATCC 9610^T (97.7%). By omitting the hypervariable regions V1 and V5 (corresponding to *E. coli* base positions 147–1490; Brosius et al., 1978), *Hafnia alvei* ATCC 13337^T showed the highest sequence similarity (96.63%) to *R. aquatilis* ATCC 33071^T. Most often isolated from fresh water, but also found in the intestine of snails and various other environmental habitats, including soils and the rhizosphere. Can occasionally be isolated from foods or human clinical specimens, including wound infections, bacteremias, feces from patients with acute gastroenteritis, and septicemia, especially from immunocompromised patients. The genus currently includes three genomospecies (*R. aquatilis* [*Rahnella* genomospecies 1], *Rahnella* genomospecies 2, and *Rahnella* genomospecies 3), which cannot be phenotypically differentiated.

The mol% G + C of the DNA is: 51–56.

Type species: **Rahnella aquatilis** Izard, Gavini, Trinel and Leclerc 1981c, 382 (Effective publication: Izard, Gavini, Trinel and Leclerc 1979, 174.)

Further descriptive information

A comparison of the total 16S rDNA sequence of the type strains of *R. aquatilis* with other representatives of the family *Enterobacteriaceae* showed highest similarity (97.7%) with the type strain of *Yersinia enterocolitica*. Eight representative strains representing all three *Rahnella* genomospecies were used for 16S rRNA gene amplification and sequencing (Brenner et al., 1998). The hypervariable regions V1 and V5 were omitted for sequence comparison resulting in a sequence that corresponded to *E. coli* base positions 147–1490 (Brosius et al., 1978). The eight strains showed sequence similarities ranging from 97.7–100% and fell in three distinct groups (Brenner et al., 1998). Three additional strains sequences already deposited in GenBank also grouped with *R. aquatilis* type strain, exhibiting 99.7–100% sequence similarity. The second group contained five strains used in the DNA–DNA relatedness experiments (Brenner et al., 1998), four of which were in genomospecies 2 and one in genomospecies 1 (*Rahnella aquatilis sensu stricto*). Two further strains, for which sequences were deposited in GenBank, were also found in this group. This observation supports the advisability of performing DNA–DNA hybridization experiments on strains showing 97% or greater sequence similarity, before considering them to belong to the same species (Brenner et al., 1998). The third group contained only strain DSM 30078, representing *Rahnella* genomospecies 3.

The phenotypic description of the genus *Rahnella* is largely based on the studies of Gavini et al. (1976a), Izard et al. (1979), Farmer (1984b), Farmer et al. (1985a), Brenner (1992a), and Brenner et al. (1998). In the study of Brenner et al. (1998), a total of 51 *Rahnella* strains were included, and the results indicated that the strains comprise three closely related species (genomospecies), which cannot be differentiated phenotypically. Their subdivision is described below (see Taxonomic Comments).

Rahnella strains grow readily on all kinds of media and no growth requirements have been described. A nutrient-rich medium (e.g., tryptone–soy agar, sheep-blood agar, or nutrient agar) gives best results. Incubation at temperatures ranging from 25–35°C results often in better growth that incubation at 37°C. The majority of strains are psychrotolerant, growing (slowly) at temperatures of 4°C. Growth was reported even at temperatures of 1°C ± 1°C (Davis and Eyles, 1992).

Members of the genus *Rahnella* ferment lactose and produce β-galactosidase (ONPG test). Acid production from D-xylose is observed in parallel with strong β-xylosidase activity. β-glucuronidase is negative in all strains. Indole production, hydrogen sulfide formation, tyrosine deaminase, urease, and DNase tests are negative. Nitrogen fixation (Berge et al., 1991; Kim et al., 1998) and mineral phosphate solubilization (Kim et al., 1998) has been reported in some strains of *R. aquatilis*. Various carbon sources, including carbohydrates, organic acids, and amino acids, are utilized as sole sources of carbon (Brenner et al., 1998).

Isoprenoid quinone Q-8 is the predominant quinone and small amounts of the menaquinone MK-8 are found in *R. aquatilis* ATCC 33071^T (P. Kämpfer, unpublished results). The fatty acid composition as determined by gas chromatographic analysis of all 51 strains used in the study of Brenner et al. (1998) differed only slightly (P. Kämpfer, unpublished results). All strains contained the fatty acids $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0\ cyclo}$, $C_{19:0\ cyclo}$, summed feature ($C_{16:1\ iso\ I}$ and/or $C_{14:0\ 3OH}$), summed feature ($C_{16:1\ \omega7c}$ and/or $C_{15:0\ iso\ 2OH}$), and summed feature ($C_{18:1\ \omega7c}$, $C_{18:1\ \omega9t}$, and/or $C_{18:1\ \omega12t}$), a fatty acid type typical of a member of the family *Enterobacteriaceae*. The dominant polyamines, as determined by high-performance liquid chromatography, were putrescine and cadaverine (Hamana, 1996). Minor amounts of diaminopropane were found in both strains studied; one strain also contained agmatine, whereas the other contained spermidine.

Members of the genus *Rahnella* are usually sensitive to aminoglycosides, cefotaxime, doxocyline, trimethoprim, gentamicin, tetracycline, and tobramycin, but resistant to chloramphenicol. Susceptibility to β-lactams differs among strains. Antimicrobial susceptibility data are summarized by Funke and Rosner (1995). They state that aminopenicillins and first-generation cephalosporins have only limited activity against *R. aquatilis*, whereas ureidopenicillins and carbapenems are more active. *R. aquatilis* is usually susceptible to aminoglycosides and quinolones. *Rahnella* produces enterobacterial common antigen (Böttger et al., 1987).

The pathogenicity of *Rahnella* in humans and animals is not clearly established. Strains isolated from humans appear to be opportunistic rather than true pathogens.

Rahnella are widely distributed in nature. They may be isolated from foods and occasionally from a wide variety of human sources. The natural habitat of *Rahnella* seems to be water, and all of the isolates of Gavini et al. (1976a) were from waters in France. Several American water isolates have also been identified as *R. aquatilis*. The study of the intestinal content of snails for the presence of *Enterobacteriaceae* (Brenner et al., 1998) resulted in the isolation of infrequently seen enterobacterial species, among them *Rahnella* spp. Rhodes et al. (1998) found *Rahnella* spp. in the intestinal content associated with 12,000-year-old mastodon remains. The study of mountain soils for psychrotrophic *Enterobacteriaceae* resulted in the isolation of *Rahnella* (Horie et al., 1985), and nitrogen-fixing strains were isolated from the rhizosphere of wheat and maize (Berge et al., 1991; Heulin et al., 1994). Heron et al. (1993) found *Rahnella* isolated among enterobacteria associated with grass and silages. The microbiolog-

ical study of prepared salad vegetables and sprouts resulted in the isolation of *Rahnella* (Geiges et al., 1990). In minced meat, fish, and milk, *Rahnella* were frequently encountered by Lindberg et al. (1998), and one fish isolate was found to contain the gene of *E. coli* heat-labile toxin (lt). Hamze et al. (1991) found *R. aquatilis* as a potential contaminant in lager beer breweries. Two strains of *Rahnella* were isolated from buckwheat seeds (Iimura and Hosono, 1996).

R. aquatilis may also occasionally occur in human clinical specimens. The first case reported was a strain from a burn wound (Farmer et al., 1985a). Maraki et al. (1994) reported a surgical wound infection with *Rahnella*. Two *Rahnella* isolates were recovered from the feces of two patients with acute gastroenteritis, one of whom was an AIDS patient (Reina and Lopez, 1996a). The strains were resistant to ampicillin, cephalothin, and cefoxitin. Funke and Rosner (1995) reported *R. aquatilis* bacteremia in an HIV-infected intravenous drug-abuser. Caraccio et al. (1994) isolated *Rahnella* from a bacteremic patient with chronic renal failure. Matsukura et al. (1996) isolated *Rahnella* aquatilis from a blood culture obtained from a case of endocarditis. *R. aquatilis* was found in the blood of a diabetic patient and a patient with laryngeal carcinoma (Oh and Tay, 1995). Both patients recovered from the infection after treatment with parenteral antibiotics. Additional cases were described by Goubau et al. (1988), Harrell et al. (1989), and Hoppe et al. (1993). Literature on *R. aquatilis* infections in humans are reviewed by Alballaa et al. (1992), Maraki et al. (1994), and Funke and Rosner (1995).

Enrichment and Isolation Procedures

Media used for the isolation of *Rahnella* are similar to those used for other members of the family *Enterobacteriaceae*. No specific selective medium is available for *Rahnella* species. As pointed out by Grimont and Grimont (1992), the requirements for a selective medium can be summarized as follows: (1) a precise and stable delineation of the taxon (genus or species); (2) known and stable common nutritional properties uncommon outside the group (genus or species); and (3) a special, in most cases clinical, or public health requirement for such a medium. Because of the low clinical significance and the presence of *Rahnella* in various habitats, the selective isolation of *Rahnella* is rarely required, except for epidemiological studies. In most cases, organisms belonging to the genus *Rahnella* are isolated with differential media not inhibitory for *Enterobacteriaceae*, such as MacConkey agar, bromothymol blue lactose agar, or phenol red lactose agar. *Rahnella* strains grow readily on nutrient-rich media, and no particular growth requirements have been described.

Maintenance Procedures

Rahnella strains can be maintained in tryptone soy agar stabs or on nutrient agar when kept at room temperature in the dark. They can be preserved by storage in broth containing 10% glycerol or in calf or bovine serum at −80°C. Lyophilization seems to be the best procedure for preservation.

Differentiation of the genus *Rahnella* from other genera

The genus *Rahnella* has no single distinguishing feature useful for differentiation from the other genera of *Enterobacteriaceae* (Table BXII.γ.193 of the *Enterobacteriaceae*). *Rahnella* does not produce a yellow pigment, is negative for lysine and ornithine decarboxylases, and is weakly positive for phenylalanine deaminase. These characters may be helpful in differentiation of *Rahnella* from the genera *Pantoea* and *Erwinia*.

Taxonomic Comments

Based on a numerical taxonomic study of *Enterobacteriaceae*, Gavini et al. (1976a) defined a new group within the family and gave it the vernacular name "group H2". Subsequently, Izard et al. (1979) used DNA–DNA hybridization to compare strains of group H2 to each other and to named species of *Enterobacteriaceae*. Based on the close relatedness within group H2 and the low relatedness to other *Enterobacteriaceae*, they proposed the new genus *Rahnella* with one species *Rahnella aquatilis*, and designated a holotype strain. The name *Rahnella* was chosen to honor Otto Rahn, the German-American microbiologist for his many contributions to systematic bacteriology and for his proposal of the family name *Enterobacteriaceae* in 1937. Because all of the isolates investigated by Izard et al. (1979) were from water, the species name *aquatilis* was chosen. The names *Rahnella* and *R. aquatilis* were effectively published (Izard et al., 1979) but were not validated in the *International Journal of Systematic Bacteriology* before 1 January 1980. They did not appear on the Approved Lists of Bacterial Names (Skerman et al., 1980), but both names have now been validly published (Izard et al., 1981c) and have standing in nomenclature. From 1979 to now, *Rahnella* was isolated from various other environmental habitats, including soils and the rhizosphere, and from food, indicating a wide distribution in nature. Furthermore, several clinical isolates were reported, in most cases from immunocompromised patients. Between 1984 and 1988, Müller et al. (1995a, b, 1996) screened the intestinal contents of snails and slugs for the presence of *Enterobacteriaceae* and found several *Rahnella* isolates in addition to the genera *Buttiauxella*, *Kluyvera*, and others. In a subsequent study 51 *Rahnella* and *Rahnella*-like organisms were subjected to an extensive DNA–DNA hybridization study. The results indicated that the strains comprised three closely related (genomo-)species within the genus, one corresponding to *R. aquatilis*. The relative binding ratios obtained from DNA–DNA hybridization studies between the *Rahnella* genomospecies are given in Table BXII.γ.259. For the two new species the vernacular names *Rahnella* genomospecies 2 and *Rahnella* genomospecies 3 were proposed (Brenner et al., 1998). It was not possible to differentiate these genomospecies on the basis of physiological and biochemical tests.

Acknowledgments

I thank Don J. Brenner for his helpful comments and critical reading of this chapter, Hans E. Müller for interesting discussions and furnishing

TABLE BXII.γ.259. Relative binding ratios of DNAs at 60°C (given as percentages/ranges of percentages) between the genomospecies of the genus *Rahnella*[a]

Test	*R. aquatilis* (*Rahnella* genomospecies 1)	*Rahnella* genomospecies 2	*Rahnella* genomospecies 3
R. aquatilis	88 (71–100)	64 (42–81)	54 (45–64)
Rahnella genomospecies 2		93 (70–100)	54 (49–61)
Rahnella genomospecies 3			100

[a]Data adapted from Brenner et al. (1998).

TABLE BXII.γ.260. Characteristics of (genomo)species of the genus *Rahnella*[a,b]

Biochemical reaction	*Rahnella aquatilis* (Genomospecies 1)	*Rahnella* (Genomospecies 2)	*Rahnella* (Genomospecies 3)
Methyl red	[+]	d	+
Voges–Proskauer	+	d	+
Citrate utilization	[+] (+)	d ([+])	−
Phenylalanine deaminase	+	d	+
Arginine dihydrolase	−	−	−
Lysine decarboxylase	−	−	−
Ornithine decarboxylase	− ([−])	−	−
Motility	d	d	−
Gelatin liquefaction (22°C)	−	− (d)	−
KCN, growth	− ([−])	−	−
Malonate	[+]	d ([+])	+
Gas from D-glucose	[+]	[+]	−
Acid from:			
D-Arabitol	−	−	+
Cellobiose	+	+	+
Dulcitol	[+] (+)	[+] (+)	−
Maltose	+	+	+
D-Mannose	+	+	+
Melibiose	+	+	+
Methyl-α-D-glucoside	−	−	−
Raffinose	+	+	+
L-Rhamnose	+	+	−
D-Sorbitol	+	+	+
Sucrose	+	+	+
D-Xylose	+	+	(+)
Glycerol	− (d)	− (d)	
Esculin hydrolysis	+	+	+
Mucate	d	−	
Tartrate	d	[+]	+
Acetate	[−]	−	−
Lipase (corn oil)	−	−	−
Lipase (Tween 80)	[+]	d	−
Utilization of:			
N-Acetyl-D-galactosamine	−	−	−
D-Fructose	[+]	d	+
Sucrose	+	+	+
Acetate	−	d	−
cis-Aconitate	d	[−]	−
trans-Aconitate	−	−	−
4-Aminobutyrate	−	−	−
DL-Lactate	+	+	−
2-Oxoglutarate	d	−	−
L-Alanine	+	d	+
L-Aspartate	+	+	+
L-Histidine	d	−	+
L-Ornithine	d	−	−
L-Phenylalanine	−	−	−
L-Proline	d	−	−
Phenylacetate	−	−	−
Hydrolysis of:			
pNP-β-D-Galactopyranoside	−	−	−
pNP-α-D-Glucopyranoside	d	−	−
pNP-β-D-Glucopyranoside	+	[−]	+
2-Deoxythymidine-5′-pNP-phosphate	−	[−]	−
L-Glutamate-γ-3-carboxy-pNA	+	[+]	−
L-Proline-pNA	[+]	−	+

[a]Symbols: +, positive for 90–100% of strains; [+], positive for 75–89% of strains; d, positive for 26–74% of strains; [−], positive for 11–25% of strains; −, positive for 0–10% of strains.

[b]Data adapted from Brenner et al. (1998). All reactions, unless otherwise stated were done at 36° ± 1°C and read after 48 h. Numbers in parentheses indicate the percentage of strains giving a positive reaction within 7 d. All strains grew at 4°C, 25°C, and 37°C, and gave positive reactions within 48 h in tests for fermentation of L-arabinose, D-galactose, D-glucose, lactose, D-mannitol, salicin, and trehalose, and in the tests for nitrate reduction to nitrite, catalase, motility at 25°C, and gelatin liquefaction at 22°C. All strains were oxidase-negative and gave negative reactions after 7 d in tests for fermentation of adonitol, erythritol, and *i*-inositol, and in tests for DNase, hydrogen sulfide, indole production, oxidase, tyrosine deaminase, urease, and yellow pigment. All strains gave positive reactions in tests for utilization of *N*-acetyl-D-glucosamine, L-arabinose, D-arbutin, D-cellobiose, D-galactose gluconate, D-glucose, D-maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, salicin, D-trehalose, D-xylose, D-mannitol, D-sorbitol, citrate, fumarate, L-malate, pyruvate, and L-serine, and positive reactions in tests for hydrolysis of pNP-β-D-galactopyranoside, pNP-β-D-xylopyranoside, bis-pNP-phosphate, pNP-phenyl-phophonate, pNP-phosphoryl choline, and L-alanine-pNA (pNP = para-nitrophenyl-; pNA = para-nitroanilide). All strains gave negative reactions in tests for utilization of adonitol, *i*-inositol, putrescine, propionate, adipate, azelate, glutarate, DL-3-hydroxybutyrate, mesaconate, suberate, β-alanine, L-leucine, L-tryptophan, 3-hydroxybenzoate, and 4-hydroxybenzoate.

with strains, and Wolfgang Ludwig for critical comments and his experience in 16S rRNA sequence analyses.

Further Reading

Brenner, D.J. 1992. Additional genera of *Enterobacteriaceae*. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes, 2nd Ed., Vol. 3, Springer-Verlag, New York. pp. 2922–2937.

Brenner, D.J., H.E. Müller, A.G. Steigerwalt, A.M. Whitney, C.M. O'Hara and P. Kämpfer. 1998. Two new *Rahnella* genomospecies that cannot be phenotypically differentiated from *Rahnella aquatilis*. Int. J. Syst. Bacteriol. *48*: 141–149.

Farmer, J.J. III 1984. Other genera of the family *Enterobacteriaceae*. *In* Krieg and Holt (Editors), Bergey's Manual of Systematic Bacteriology, 1st Ed., Vol. 1, The Williams & Wilkins Co, Baltimore. pp. 506–516.

Gavini, F., C. Ferragut, B. Lefebre and H. Leclerc. 1976. Étude taxonomique d'enterobactéries appartenant ou apparentées au genre *Enterobacter*. Ann. Inst. Pasteur Microbiol. *127B*: 317–335.

Izard, D., F. Gavini, P.A. Trinel and H. Leclerc. 1979. *Rahnella aquatilis*, nouveau membre de la famille des *Enterobacteriaceae*. Ann. Inst. Pasteur Microbiol. *130A*: 163–177.

List of species of the genus Rahnella

1. **Rahnella aquatilis** Izard, Gavini, Trinel and Leclerc 1981c, 382[VP] (Effective publication: Izard, Gavini, Trinel and Leclerc 1979, 174.)
a.qua′ti.lis. L. adj. *aquatilis* living in water.

   Represents *Rahnella* genomospecies 1 of the study of Brenner et al. (1998). The characteristics are as described for the genus. Detailed characteristics are given in Table BXII.γ.260.

   Occurs in freshwater, but also in environmental habitats like soil. May occasionally be isolated from human clinical specimens; in most cases from immunocompromised hosts. Clinical significance is unknown. Mean DNA–DNA relatedness among the 21 strains studied by Brenner et al. (1998), including 9 of the 10 original water isolates described by Izard et al. (1979) was 88% in 60°C reactions (range 72–100%). Mean relatedness in 75°C reactions was 81% (range 61–100%).

   The mol% G + C of the DNA is: 51–56 (T_m).
   Type strain: 133, ATCC 33071, CIP 78-65, DSM 4594.
   GenBank accession number (16S rRNA): AJ233426.

Other Organisms

1. *Rahnella* genomospecies 2.

   This species was proposed on the basis on DNA–DNA hybridization experiments and comprises 30 strains (Brenner et al., 1998). Phenotypically it is indistinguishable from *Rahnella aquatilis*. A combination of the utilization tests with L-histidine, L-ornithine, and L-proline (Table BXII.γ.260; Brenner et al., 1998) may be helpful in differentiation from *Rahnella aquatilis*, but does not allow a clear (genomo)-species identification. All 30 strains studied by Brenner et al. (1998) were 83% or more related in 60°C reactions (range 70–100%). In 75°C reactions the strains were 74% or more related, with one exception showing only 68% relatedness to the labeled reference organism.

   Strains were isolated from water, intestinal contents of snails, and clinical material, including stored donated human blood.

   Deposited strain: SM S7/1-576.

2. *Rahnella* genomospecies 3.

   This species was also proposed based on DNA–DNA hybridization experiments (Brenner et al., 1998) and is represented by a single strain: DSM 30078 (isolated from minced meat). Phenotypically it can be separated from *Rahnella aquatilis* and *Rahnella* genomospecies 2 (Table BXII.γ.260), but without results from different strains one cannot determine whether the results are strain- or species-specific.

   Deposited strain: DSM 30078.

Genus XXXII. **Saccharobacter** *Yaping, Xiaoyang and Jiaqu 1990, 412[VP]*

Don J. Brenner

Sac.cha.ro.bac′ter. L. n. *saccharum* sugar; M.L. n. *bacter* a rod; M.L. masc. n. *Saccharobacter* a sugar rod.

Small straight rods, 0.5–0.9 × 1.0–1.0 μm. **Gram negative, motile by peritrichous flagella.** Facultatively anaerobic. Chemoorganotrophic. Colonies on glucose-yeast extract agar are opaque and milky white, smooth, and low convex with entire margins. Oxidase negative, catalase positive. Nonpigmented. Nonsporeforming. No lipid granules present. Optimal growth temperature range is from 30–46°C. **D-glucose is fermented with the production of ethanol, CO_2, and small amounts of acids, but no hydrogen. Indole negative, methyl red positive, Voges–Proskauer test positive, citrate positive**. H_2S production, gelatin hydrolysis, urease, nitrogen reduction, lysine decarboxylase, and ornithine decarboxylase are negative; arginine dihydrolase is delayed positive (4 d). Acid is produced from D-glucose, L-arabinose, esculin, D-fructose, D-galactose, maltose, D-mannitol, melibiose, L-rhamnose, L-sorbose, starch, sucrose, trehalose, D-xylose. Lactose fermentation is delayed positive after 9 d. Dulcitol, gluconate, *myo*-inositol, and raffinose are not fermented. Malonate is used as a sole carbon source; ammonium sulfate, yeast extract, urea, phenylalanine, glutamine, and tryptone serve as sole nitrogen sources, but sodium glutamate does not. Produces β-galactosidase and grows in the presence of KCN. **Grows in the presence of 35% D-glucose and 6% NaCl, but not 40% D-glucose or 8% NaCl.** 16S rDNA sequence has not been determined.

The mol% G + C of the DNA is: 63.5.

Type species: **Saccharobacter fermentatus** Yaping, Xiaoyang, and Jiaqu 1990, 412.

Further descriptive information

Carbohydrates are presumably degraded by the Embden-Meyerhof-Parnas pathway, as indicated by the absence of 6-phos-

phogluconate dehydratase and 2-keto-3-deoxygluconate-6-phosphate aldolase and the presence of fructose diphosphate aldolase. Degrades 1 mol of glucose to approximately 2 mol of ethanol and 2 mol of CO_2.

Maintenance Procedures

Cultures are grown at 30°C and maintained on glucose-peptone-yeast extract agar. Lyophilization is used for long-term storage.

Differentiation of the genus *Saccharobacter* from other genera

Saccharobacter is easily differentiated from *Zymomonas*, another ethanol-producing genus. *Saccharobacter* is peritrichously flagellated, whereas *Zymomonas* has polar flagella. *Zymomonas* ferments only D-glucose, fructose, and sucrose, whereas *Saccharobacter* ferments a number of other carbohydrates (see above). *Zymomonas* degrades sugars by the Entner-Duoderoff pathway, while *Saccharobacter* ferments sugars by the Embden-Meyerhof-Parnas pathway. *Saccharobacter* can easily be differentiated from various species of *Enterobacteriaceae* based on its indole, methyl red, Voges–Proskauer, and citrate (IMViC) reactions, together with its negative nitrate reductase reaction, its positive phenylalanine deaminase reaction, and its sugar fermentation pattern (see chapter on *Enterobacteriaceae*).

Taxonomic Comments

Since 16S rDNA sequencing has not been done on the type species, it is impossible to accurately place the genus *Saccharobacter* phylogenetically. *Saccharobacter* appears to be phenotypically similar to members of the family *Enterobacteriaceae* based on a number of key characteristics. These include negative oxidase and positive catalase reactions, peritrichous flagella, facultatively anaerobic metabolism, and fermentation of D-glucose and other carbohydrates. It differs from *Enterobacteriaceae* in the end products of fermentation, which in *Enterobacteriaceae* are mixed acids or 2,3-butanediol, with no more then 0.5 mol of ethanol from 1 mol of D-glucose. *Saccharobacter* also differs from most, if not all, members of *Enterobacteriaceae* by the high mol% G + C content.

Further Reading

Yaping, J., L. Xiaoyang and Y. Jiaqi. 1990. *Saccharobacter fermentatus* gen. nov., sp. nov., a new ethanol-producing bacterium. Int. J. Syst. Bacteriol. *40*: 412–414.

List of species of the genus Saccharobacter

1. **Saccharobacter fermentatus** Yaping, Xiaoyang and Jiaqu 1990, 412[VP]
   fer.men.ta'tus. M.L. adj. *fermentatus* fermentative.

   The characteristics are those given for the genus. All extant strains were isolated from squeezed leaf juice of agave. Isolated from squeezed leaf juice of agave in Wuhan, People's Republic of China.

   The mol% G + C of the DNA is: 63.5 (T_m).
   Type strain: WVB8512.

Genus XXXIII. **Salmonella** *Lignières 1900, 389*[AL]

Michel Y. Popoff and Léon E. Le Minor

Sal.mon.el'la. M.L. *-ella* dim. ending; M.L. fem. n. *Salmonella* named after D.E. Salmon, an American bacteriologist.

Straight rods, 0.7–1.5 × 2.0–5.0 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. **Usually motile** (peritrichous flagella). Facultatively anaerobic. Colonies are generally 2–4 mm in diameter. Nitrates are reduced to nitrites. **Gas is usually produced from D-glucose.** Hydrogen sulfide is usually produced on triple-sugar iron agar. Indole negative. **Citrate is usually utilized as a sole carbon source.** Lysine and ornithine decarboxylase (Møller's) reactions are usually positive. Urease negative. Phenylalanine and tryptophan are not oxidatively deaminated. Sucrose, salicin, inositol, and amygdalin are usually not fermented. Lipase and deoxyribonuclease are not produced. Pathogenic for humans, causing enteric fevers, gastroenteritis, and septicemia; may also infect many animal species besides humans. Some serovars are strictly host-adapted.

The mol% G + C of the DNA is: 50–53.

Type species: **Salmonella choleraesuis** (Smith 1894) Weldin 1927, 155 (*Bacillus cholerae suis* Smith 1894, 9.)

Further descriptive information

Although most salmonellae are motile, the serovar Gallinarum (or serovar Pullorum) is always nonmotile.

Certain *Salmonella* serovars, such as serovar Abortusovis, may form unusually small colonies (~1 mm diameter), whereas most types form larger colonies (2–4 mm).

Most salmonellae are aerogenic. However, serovar Typhi, an important exception, never produces gas. Anaerogenic variants of normally gas-producing *Salmonella* serovars may occur; this is particularly common with serovar Dublin.

Hydrogen sulfide is produced by most salmonellae, but a few serovars do not produce it (e.g., most strains of serovar Paratyphi A and some strains of serovar Choleraesuis).

Citrate is generally utilized by salmonellae, but some serovars do not use it (particularly host-adapted serovars such as serovar Typhi and serovar Paratyphi A).

The lysine decarboxylase reaction (Møller's) is positive for most salmonellae, but an important exception is serovar Paratyphi A. Most salmonellae are also positive for ornithine decarboxylase reaction (Møller's), but serovar Typhi is negative.

Other biochemical characteristics of the genus are indicated in Tables BXII.γ.193, BXII.γ.194, and BXII.γ.196 in the chapter on the family *Enterobacteriaceae*. Subdivision of the genus *Salmonella* into species and subspecies (Le Minor and Popoff, 1987; Reeves et al., 1989a) based on biochemical characteristics is shown in Table BXII.γ.261. Kauffmann (1960, 1963a, b, 1964) subdivided the genus *Salmonella* into "subgenera" (see Taxonomic Comments). These subdivisions correspond more closely to species or subspecies in other groups of bacteria, but whatever rank is assigned to them, the worthiness of these subdivisions was confirmed by Rohde (1965, 1966, 1967).

TABLE BXII.γ.261. Differential characteristics of the *Salmonella* species and subspecies[a]

Characteristic	*S. enterica* subsp. *enterica*	*S. enterica* subsp. *arizonae*	*S. enterica* subsp. *diarizonae*	*S. enterica* subsp. *houtenae*	*S. enterica* subsp. *indica*	*S. enterica* subsp. *salamae*	*S. bongori*
Dulcitol	+	−	−	−	d	+	+
ONPG (2 h)	−	+	+	−	d	−	+
Malonate	−	+	+	−	−	+	−
Gelatinase	−	+	+	+	+	+	−
Sorbitol	+	+	+	+	−	+	+
Culture with KCN	−	−	−	+	−	−	+
L(+)-Tartrate[b]	+	−	−	−	−	−	−
Galacturonate	−	−	+	+	+	+	+
γ-Glutamyltransferase	+[c]	−	+	+	+	+	+
β-Glucuronidase	d	−	+	−	d	d	−
Mucate	+	+	− (70%)	−	+	+	+
Salicine	−	−	−	+	−	−	−
Lactose	−	− (75%)	+ (75%)	−	d	−	−
Lysis by phage O1	+	−	+	−	+	+	d
Habitat:							
Warm-blooded animals	+						
Cold-blooded animals		+	+	+	+	+	+

[a]Symbols: + , positive for 90% or more of strains in 1–2 days; d, positive for 11–89% of strains in 1–2 days; −, positive for 0–10% of strains in 1–2 days, unless otherwise indicated in the table. The temperature for all reactions is 37°C.

[b]D-tartrate.

[c] Typhimurium d, Dublin −.

Division into serovars The antigenic formulae of *Salmonella* serovars are listed in the Kauffmann–White scheme (Table BXII.γ.262). They are composed of numbers and letters given to the different O (somatic), Vi (capsular), and H (flagellar) antigens. Only those antigens of primary diagnostic importance are indicated in the Kauffmann–White scheme. Antigenic formulae (for example 6,7,[Vi]:c:1,5) represent the O antigenic factors, the Vi capsular antigen when present, the first phase of the H antigen, and the second phase of the H antigen, respectively. Those formulae with major O antigenic factors in common are collected into an O group and arranged alphabetically by the first phase of the H antigen within the group.

Lysogenization by certain converting phages may produce changes in the O antigenic formulae of salmonellae. In groups O:2, O:4, and O:9, presence of O:1 antigenic factor is associated with lysogenization (Iseki and Kashiwagi, 1955, 1957; Zinder, 1957; Stocker, 1958), but presence or absence of this factor in strains of these groups does not change the name of the serovar (for example, serovar Typhimurium applies to both O:1-positive and O:1-negative strains). Factors associated with phage conversion are underlined in the Kauffmann–White scheme. Converting phages of *Salmonella* are identical in morphology (Vieu et al., 1965), but their action is limited to certain O groups and they are serologically different from one another (Le Minor, 1968).

The specificities of O factors in *Salmonella* are determined by the composition and structure of the polysaccharides. Specificity is modified during smooth to rough mutation and by bacteriophage conversions (see reviews by Lüderitz et al., 1971; Stocker and Mäkelä, 1971, 1978). The only difference between the 4,12 and the 9,12 O-specific repeating units is in the di-deoxyhexose branch unit attached to the mannose, which is abequose in O:4,12 and tyvelose in O:9,12. In the conversion of O:3,10 to O:3,15, the terminal acetyl radical of the chain is suppressed and the α-lineage between galactose and mannose is transformed into a β-linkage. Other modifications of the specificity of O antigens may occur after mutation(s), resulting in new specificities called T1 and T2 by Kauffmann (1956) or in different R types (reviewed by Lüderitz et al., 1971; Stocker and Mäkelä, 1971, 1978).

Surface antigens, commonly observed in other members of the *Enterobacteriaceae* family (e.g., *Escherichia coli* and *Klebsiella*), may be found in some *Salmonella* serovars. Surface antigens in *Salmonella* may mask O antigens, and the culture will not agglutinate with O antisera. The Vi antigen is a surface antigen found mainly in serovar Typhi and serovar Paratyphi C, as well as in a few strains of serovar Dublin. Heating at 100°C generally solubilizes Vi antigen, and heat-treated bacteria agglutinate with proper O antisera. Biogenesis of the Vi polysaccharide is governed by a set of genes located at the *viaB* locus, which is specific to Vi-expressing strains. Expression of Vi antigen is controlled by two two-component regulatory systems, OmpR-EnvZ and RcsB-RcsC (reviewed by Virlogeux-Payant and Popoff, 1996). One signal for this complex regulatory control was shown to be osmolarity (Pickard et al., 1994).

Subdivision of serovars Biovars are different sugar fermentation patterns shown by strains of the same serovar. They are determined by the presence or absence of enzymes and hence are genetically determined. Biovars may serve as markers and be of interest epidemiologically (for example, the xylose-positive and xylose-negative character of serovar Typhi).

Phagovars are determined by the sensitivity of cultures to a series of bacteriophages at appropriate dilutions. Phage typing of serovar Typhi and other salmonellae that possess the Vi antigen (serovar Paratyphi C and a few strains of serovar Dublin) is based on a series of adapted phages from phage Vi II of Craigie and Yen (1938). Phage typing of serovar Paratyphi B (Felix and Callow, 1943) and serovar Typhimurium (Anderson, 1964) uses a different series of phages. Analogous methods have been proposed for other serovars of *Salmonella*, some of them making use of the lysogenicity of the strains.

Other subdivisions of serovars may be based on production of, or on sensitivity to, bacteriocins, and on resistance to antibiotics.

TABLE BXII.γ.262. Antigenic formulae of the serovars of the genus *Salmonella*: the Kauffmann–White scheme[a]

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Group O:2 (A)			
Paratyphi A	1,2,12	a	[1,5]
Nitra	2,12	g,m	
Kiel	1,2,12	g,p	
Koessen	2,12	l,v	1,5
Group O:4(B)			
Kisangani	1,4,[5],12	a	1,2
Hessarek	4,12,27	a	1,5
Fulica	4,[5],12	a	
Arechavaleta	4,[5],12	a	1,7
Bispebjerg	1,4,[5],12	a	e,n,x
Tinda	1,4,12, 27	a	e,n,z_{15}
II	1,4,[5],12, 27	a	e,n,x
Huettwillen	1,4,12	a	l,w
Nakuru	1,4,12, 27	a	z_6
II	1,4,12, 27	a	z_{39}
Paratyphi B	1,4,[5],12	b	1,2
Limete	1,4,12, 27	b	1,5
II	4,12	b	1,5
Canada	4,12,27	b	1,6
Uppsala	1,4,12, 27	b	1,7
Abony	1,4,[5],12, 27	b	e,n,x
II	1,4,12, 27	b	[e,n,x]
Wagenia	1,4,12, 27	b	e,n,z_{15}
Wien	1,4,12, 27	b	l,w
Tripoli	1,4,12, 27	b	z_6
Schleissheim	4,12,27	b	
Legon	1,4,12, 27	c	1,5
Abortusovis	4,12	c	1,6
Altendorf	4,12,27	c	1,7
Bissau	4,12	c	e,n,x
Jericho	1,4,12, 27	c	e,n,z_{15}
Hallfold	1,4,12, 27	c	l,w
Bury	4,12,27	c	z_6
Stanley	1,4,[5],12, 27	d	1,2
Eppendorf	1,4,12, 27	d	1,5
Brezany	1,4,12, 27	d	1,6
Schwarzengrund	1,4,12, 27	d	1,7
II	4,12	d	e,n,x
Sarajane	1,4,[5],12, 27	d	e,n,x
Duisburg	1,4,12, 27	d	e,n,z_{15}
Mons	1,4,12, 27	d	l,w
Ayinde	1,4,12, 27	d	z_6
Saintpaul	1,4,[5],12	e,h	1,2
Reading	1,4,[5],12	e,h	1,5
Eko	4,12	e,h	1,6
Kaapstad	4,12	e,h	1,7
Chester	1,4,[5],12	e,h	e,n,x
Sandiego	4,[5],12	e,h	e,n,z_{15}
II	4,12	e,n,x	1,2,7
II	1,4,12, 27	e,n,x	1,[5],7
Derby	1,4,[5],12	f,g	[1,2]
Agona	1,4,12	f,g,s	[1,2]
II	1,4,[5],12	f,g,t	z_6:z_{42}
Essen	4,12	g,m	
Hato	1,4,[5],12	g,m,s	
II	1,4,12, 27	g,[m],[s],t	e,n,x
II	1,4,12, 27	g,[m],t	[1,5]
II	4,12	g,m,t	z_{39}
California	4,12	g,m,t	[z_{67}]
Kingston	1,4,[5],12, 27	g,s,t	[1,2]
Budapest	1,4,12, 27	g,t	
Travis	4,[5],12	g,z_{51}	1,7
Tennyson	4,5,12	g,z_{51}	e,n,z_1
II	4,12	g,z_{62}	
Banana	1,4,[5],12	m,t	[1,5]
Madras	4,[5],12	m,t	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Typhimurium	1,4,[5],12	i	1,2
Lagos	1,4,[5],12	i	1,5
Agama	4,12	i	1,6
Farsta	4,12	i	e,n,x
Tsevie	4,12	i	e,n,z_{15}
Gloucester	1,4,12, 27	i	l,w
Tumodi	1,4,12	i	z_6
II	4,12,27	i	z_{35}
Massenya	1,4,12, 27	k	1,5
Neumuenster	1,4,12, 27	k	1,6
II	1,4,12, 27	k	1,6
Ljubljana	4,12,27	k	e,n,x
Texas	4,[5],12	k	e,n,z_{15}
Fyris	4,[5],12	l,v	1,2
Azteca	4,[5],12,27	l,v	1,5
Clackamas	4,12	l,v	1,6
Bredeney	1,4,12, 27	l,v	1,7
Kimuenza	1,4,12, 27	l,v	e,n,x
II	1,4,12, 27	l,v	e,n,x
Brandenburg	1,4,[5],12, 27	l,v	e,n,z_{15}
II	1,4,12, 27	l,v	z_{39}
Mono	4,12	l,w	1,5
Togo	4,12	l,w	1,6
II	4,12	l,w	e,n,x
Ayton	1,4,12, 27	l,w	z_6
Kunduchi	1,4,[5],12, 27	l,[z_{13}],[z_{28}]	1,2
Tyresoe	1,4,12, 27	l,[z_{13}],z_{28}	1,5
Haduna	4,12	l,z_{13},[z_{28}]	1,6
Kubacha	1,4,12, 27	l,z_{13},z_{28}	1,7
Kano	1,4,12, 27	l,z_{13},z_{28}	e,n,x
Vom	1,4,12, 27	l,z_{13},z_{28}	e,n,z_{15}
Reinickendorf	4,12	l,z_{28}	e,n,x
II	4,12	l,z_{28}	
Heidelberg	1,4,[5],12	r	1,2
Bradford	4,12,27	r	1,5
Winneba	4,12	r	1,6
Remo	1,4,12, 27	r	1,7
Bochum	4,[5],12	r	l,w
Southampton	1,4,12, 27	r	z_6
Drogana	1,4,12, 27	r,i	e,n,z_{15}
Africana	4,12	r,i	l,w
Coeln	1,4,[5],12	y	1,2
Trachau	4,12,27	y	1,5
Finaghy	4,12	y	1,6
Teddington	1,4,12, 27	y	1,7
Ball	1,4,12, 27	y	e,n,x
Jos	1,4,12, 27	y	e,n,z_{15}
Kamoru	4,12,27	y	z_6
Shubra	4,[5],12	z	1,2
Kiambu	1,4,12	z	1,5
II	1,4,12, 27	z	1,5
Loubomo	4,12	z	1,6
Indiana	1,4,12	z	1,7
II	4,12	z	1,7
Neftenbach	4,12	z	e,n,x
II	1,4,12, 27	z	e,n,x
Koenigstuhl	1,4,[5],12	z	e,n,z_{15}
Preston	1,4,12	z	l,w
Entebbe	1,4,12, 27	z	z_6
II	4,12	z	z_{39}
Stanleyville	1,4,[5],12, 27	z_4,z_{23}	[1,2]
Vuadens	4,12,27	z_4,z_{23}	z_6
Kalamu	4,[5],12	z_4,z_{24}	[1,5]
Haifa	1,4,[5],12	z_{10}	1,2
Ituri	1,4,12	z_{10}	1,5
Tudu	4,12	z_{10}	1,6
Albert	4,12	z_{10}	e,n,x
Tokoin	4,12	z_{10}	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Mura	1,4,12	z_{10}	l,w
Fortune	1,4,12, 27	z_{10}	z_6
Vellore	1,4,12, 27	z_{10}	z_{35}
Brancaster	1,4,12, 27	z_{29}	
II	1,4,12	z_{29}	e,n,x
Pasing	4,12	z_{35}	1,5
Tafo	1,4,12, 27	z_{35}	1,7
Sloterdijk	1,4,12, 27	z_{35}	z_6
Yaounde	1,4,12, 27	z_{35}	e,n,z_{15}
Tejas	4,12	z_{36}	
Wilhelmsburg	1,4,[5],12, 27	z_{38}	[e,n,z_{15}]
II	1,4,12, 27	z_{39}	1,[5],7
Thayngen	1,4,12, 27	z_{41}	1,(2),5
Maska	1,4,12, 27	z_{41}	e,n,z_{15}
Abortusequi	4,12		e,n,x
Group O:7 (C1)[d]			
Sanjuan	6,7	a	1,5
II	6,7,14	a	1,5
Umhlali	6,7	a	1,6
Austin	6,7	a	1,7
Oslo	6,7,14	a	e,n,x
Denver	6,7	a	e,n,z_{15}
Coleypark	6,7,14	a	l,w
Damman	6,7	a	z_6
II	6,7	a	z_6
II	6,7	a	z_{42}
Brazzaville	6,7	b	1,2
Edinburg	6,7,14	b	1,5
Adime	6,7	b	1,6
Koumra	6,7	b	1,7
Lockleaze	6,7,14	b	e,n,x
Georgia	6,7	b	e,n,z_{15}
II	6,7	b	[e,n,x]:z_{42}
Ohio	6,7,14	b	l,w
Leopoldville	6,7	b	z_6
Kotte	6,7	b	z_{35}
II	6,7	b	z_{39}
Hissar	6,7,14	c	1,2
Paratyphi C	6,7,[Vi]	c	1,5
Choleraesuis	6,7	c	1,5
Typhisuis	6,7	c	1,5
Birkenhead	6,7	c	1,6
Schwabach	6,7	c	1,7
Namibia	6,7	c	e,n,x
Kaduna	6,7,14	c	e,n,z_{15}
Kisii	6,7	d	1,2
Isangi	6,7,14	d	1,5
Kivu	6,7	d	1,6
Kambole	6,7	d	1,[2],7
Amersfoort	6,7,14	d	e,n,x
Gombe	6,7,14	d	e,n,z_{15}
Livingstone	6,7,14	d	l,w
Wil	6,7	d	l,z_{13},z_{28}
Nieukerk	6,7,14	d	z_6
II	6,7	d	z_{42}
Larochelle	6,7	e,h	1,2
Lomita	6,7	e,h	1,5
Norwich	6,7	e,h	1,6
Nola	6,7	e,h	1,7
Braenderup	6,7,14	e,h	e,n,z_{15}
II	6,7	e,n,x	1,6:z_{42}
Rissen	6,7,14	f,g	
Eingedi	6,7	f,g,t	1,2,7
Afula	6,7	f,g,t	e,n,x
Montevideo	6,7,14	g,m,[p],s	[1,2,7]
II	6,7	g,m,[s],t	e,n,x
II	6,7	(g),m,[s],t	1,5
II	6,7	g,[m],s,t	[z_{42}]

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Othmarschen	6,7,14	g,m,[t]	
Menston	6,7	g,s,[t]	[1,6]
II	6,7	g,t	e,n,x:z_{42}
Riggil	6,7	g,(t)	
Alamo	6,7	g,z_{51}	1,5
IV	6,7	g,z_{51}	
Haelsingborg	6,7	m,p,t,[u]	
Winston	6,7	m,t	1,6
Oakey	6,7	m,t	z_{64}
II	6,7	m,t	
Oranienburg	6,7,14	m,t	[z_{57}]
Augustenborg	6,7,14	i	1,2
Oritamerin	6,7	i	1,5
Garoli	6,7	i	1,6
Lika	6,7	i	1,7
Athinai	6,7	i	e,n,z_{15}
Norton	6,7	i	l,w
Stuttgart	6,7,14	i	z_6
Galiema	6,7,14	k	1,2
Thompson	6,7,14	k	1,5
Daytona	6,7	k	1,6
Baiboukoum	6,7	k	1,7
Singapore	6,7	k	e,n,x
Escanaba	6,7	k	e,n,z_{15}
IIIb	6,7	(k)	z:[z_{54}]
II	6,7	k	[z_6]
Concord	6,7	l,v	1,2
Irumu	6,7	l,v	1,5
Mkamba	6,7	l,v	1,6
Kortrijk	6,7	l,v	1,7
Bonn	6,7	l,v	e,n,x
Potsdam	6,7,14	l,v	e,n,z_{15}
Gdansk	6,7,14	l,v	z_6
Coromandel	6,7	l,v	z_{35}
IIIb	6,7	l,v	z_{53}
Gabon	6,7	l,w	1,2
Colorado	6,7	l,w	1,5
II	6,7	l,w	1,5,7
II	6,7	l,w	z_{42}
Nessziona	6,7	l,z_{13}	1,5
Kenya	6,7	l,z_{13}	e,n,x
Neukoelln	6,7	l,z_{13},[z_{28}]	e,n,z_{15}
Makiso	6,7	l,z_{13},z_{28}	z_6
Strathcona	6,7	l,z_{13},z_{28}	1,7
II	6,7	l,z_{28}	1,5:[z_{42}]
II	6,7	l,z_{28}	e,n,x
II	6,7	l,z_{28}	z_6
Virchow	6,7	r	1,2
Infantis	6,7,14	r	1,5
Nigeria	6,7	r	1,6
Colindale	6,7	r	1,7
Papuana	6,7	r	e,n,z_{15}
Grampian	6,7	r	l,w
Richmond	6,7	y	1,2
Bareilly	6,7,14	y	1,5
Oyonnax	6,7	y	1,6
Gatow	6,7	y	1,7
Hartford	6,7	y	e,n,x:[z_{67}]
Mikawasima	6,7,14	y	e,n,z_{15}
Chile	6,7	z	1,2
Poitiers	6,7	z	1,5
II	6,7	z	1,5
Oakland	6,7	z	1,6,[7]
Cayar	6,7	z	e,n,x
II	6,7	z	e,n,x
Businga	6,7	z	e,n,z_{15}
Bruck	6,7	z	l,w
II	6,7	z	z_6

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
II	6,7	z	z_{39}
II	6,7	z	z_{42}
Obogu	6,7	z_4,z_{23}	1,5
Planckendael	6,7	z_4,z_{23}	1,6
Aequatoria	6,7	z_4,z_{23}	e,n,z_{15}
Goma	6,7	z_4,z_{23}	z_6
IV	6,7	z_4,z_{23}	
II	6,7	z_4,z_{24}	z_{42}
Somone	6,7	z_4,z_{24}	
IV	6,7	z_4,z_{24}	
II	6,7	z_6	1,7
Menden	6,7	z_{10}	1,2
Inganda	6,7	z_{10}	1,5
Eschweiler	6,7	z_{10}	1,6
Ngili	6,7	z_{10}	1,7
Djugu	6,7	z_{10}	e,n,x
Mbandaka	6,7,14	z_{10}	e,n,z_{15}
Jerusalem	6,7,14	z_{10}	l,w
Redba	6,7	z_{10}	z_6
Omuna	6,7	z_{10}	z_{35}
Tennessee	6,7,14	z_{29}	[1,2,7]
II	6,7	z_{29}	[z_{42}]
Tienba	6,7	z_{35}	1,6
Palime	6,7	z_{35}	e,n,z_{15}
Tampico	6,7	z_{36}	e,n,z_{15}
II	6,7	z_{36}	z_{42}
IV	6,7	z_{36}	
Rumford	6,7	z_{38}	1,2
Lille	6,7,14	z_{38}	
IIIb	6,7,14	z_{39}	1,2
II	6,7	z_{39}	1,5,7
VI	6,7	z_{41}	1,7
Hillsborough	6,7	z_{41}	l,w
Tamilnadu	6,7	z_{41}	z_{35}
II	6,7	z_{42}	1,7
Bulovka	6,7	z_{44}	
II	6,7		1,6
Group O:8 (C2–C3)[e]			
Be	8,20	a	[z_6]
Valdosta	6,8	a	1,2
Doncaster	6,8	a	1,5
Curacao	6,8	a	1,6
Nordufer	6,8	a	1,7
Narashino	6,8	a	e,n,x
II	6,8	a	e,n,x
Leith	6,8	a	e,n,z_{15}
II	6,8	a	z_{39}
II	6,8	a	z_{52}
Djelfa	8	b	1,2
Skansen	6,8	b	1,2
Korbol	8,20	b	1,5
Nagoya	6,8	b	1,5
II	6,8	b	1,5
Stourbridge	6,8	b	1,6
Sanga	8	b	1,7
Eboko	6,8	b	1,7
Konstanz	8	b	e,n,x
Gatuni	6,8	b	e,n,x
Shipley	8,20	b	e,n,z_{15}
Presov	6,8	b	e,n,z_{15}
Bukuru	6,8	b	l,w
Tounouma	8,20	b	z_6
Banalia	6,8	b	z_6
Wingrove	6,8	c	1,2
Utah	6,8	c	1,5
Bronx	6,8	c	1,6
Belfast	6,8	c	1,7
Alexanderpolder	8	c	l,w
Santiago	8,20	c	e,n,x
Belem	6,8	c	e,n,x
Quiniela	6,8	c	e,n,z_{15}
Tado	8,20	c	z_6
Virginia	8	d	1,2
Muenchen	6,8	d	1,2:[z_{67}]
Yovokome	8,20	d	1,5
Manhattan	6,8	d	1,5
Portanigra	8,20	d	1,7
Dunkwa	6,8	d	1,7
Sterrenbos	6,8	d	e,n,x
Herston	6,8	d	e,n,z_{15}
Labadi	8,20	d	z_6
II	6,8	d	z_6:z_{42}
Bardo	8	e,h	1,2
Newport	6,8,20	e,h	1,2:[z_{67}]
Ferruch	8	e,h	1,5
Kottbus	6,8	e,h	1,5
Cremieu	6,8	e,h	1,6
Atakpame	8,20	e,h	1,7
Tshiongwe	6,8	e,h	e,n,z_{15}
Rechovot	8,20	e,h	z_6
Sandow	6,8	f,g	e,n,z_{15}
II	6,8	f,g,m,t	[e,n,x]
Emek	8,20	g,m,s	
Chincol	6,8	g,m,[s]	[e,n,x]
II	6,8	g,m,t	1,7
Reubeuss	8,20	g,m,t	
Alminko	8,20	g,s,t	
Nanergou	6,8	g,s,t	
Yokoe	8,20	m,t	
II	6,8	m,t	1,5
II	6,8	m,t	e,n,x
Bassa	6,8	m,t	
Lindenburg	6,8	i	1,2
Bargny	8,20	i	1,5
Takoradi	6,8	i	1,5
Warnow	6,8	i	1,6
Malmoe	6,8	i	1,7
Bonariensis	6,8	i	e,n,x
Aba	6,8	i	e,n,z_{15}
Magherafelt	8,20	i	l,w
Cyprus	6,8	i	l,w
Kentucky	8,20	i	z_6
Kallo	6,8	k	1,2
Haardt	8	k	1,5
Blockley	6,8	k	1,5
Schwerin	6,8	k	e,n,x
Charlottenburg	6,8	k	e,n,z_{15}
Pakistan	8	l,v	1,2
Litchfield	6,8	l,v	1,2
Loanda	6,8	l,v	1,5
Amherstiana	8	l,v	1,6
Manchester	6,8	l,v	1,7
Holcomb	6,8	l,v	e,n,x
II	6,8	l,v	e,n,x
Edmonton	6,8	l,v	e,n,z_{15}
Fayed	6,8	l,w	1,2
II	6,8	l,w	z_6:z_{42}
Hiduddify	6,8	l,z_{13},z_{28}	1,5
Breukelen	6,8	l,z_{13},[z_{28}]	e,n,z_{15}
II	6,8	l,z_{28}	e,n,x
Bsilla	6,8	r	1,2
Hindmarsh	8,20	r	1,5
Bovismorbificans	6,8,20	r,[i]	1,5
Noya	8	r	1,7
Akanji	6,8	r	1,7
Cocody	8,20	r,i	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Hidalgo	6,8	r,[i]	e,n,z_{15}
Brikama	8,20	r,[i]	l,w
Goldcoast	6,8	r	l,w
Altona	8,20	r,[i]	z_6
Giza	8,20	y	1,2
Brunei	8,20	y	1,5
Tananarive	6,8	y	1,5
Bulgaria	6,8	y	1,6
II	6,8	y	1,6:z_{42}
Alagbon	8	y	1,7
Inchpark	6,8	y	1,7
Sunnycove	8	y	e,n,x
Daarle	6,8	y	e,n,x
Praha	6,8	y	e,n,z_{15}
Kraligen	8,20	y	z_6
Benue	6,8	y	l,w
Sindelfingen	8,20	y	l,w
Mowanjum	6,8	z	1,5
II	6,8	z	1,5
Phaliron	8	z	e,n,z_{15}
Kalumburu	6,8	z	e,n,z_{15}
Kuru	6,8	z	l,w
Daula	8,20	z	z_6
Bellevue	8	z_4,z_{23}	1,7
Lezennes	6,8	z_4,z_{23}	1,7
Breda	6,8	z_4,z_{23}	e,n,x
Chailey	6,8	z_4,z_{23}	e,n,z_{15}
Dabou	8,20	z_4,z_{23}	l,w
Corvallis	8,20	z_4,z_{23}	[z_6]
Albany	8,20	z_4,z_{24}	
Duesseldorf	6,8	z_4,z_{24}	
Tallahassee	6,8	z_4,z_{32}	
Bazenheid	8,20	z_{10}	1,2
Zerifin	6,8	z_{10}	1,2
Paris	8,20	z_{10}	1,5
Mapo	6,8	z_{10}	1,5
Cleveland	6,8	z_{10}	1,7
Istanbul	8	z_{10}	e,n,x
Hadar	6,8	z_{10}	e,n,x
Chomedey	8,20	z_{10}	e,n,z_{15}
Glostrup	6,8	z_{10}	e,n,z_{15}
Remiremont	8,20	z_{10}	l,w
Molade	8,20	z_{10}	z_6
Wippra	6,8	z_{10}	z_6
II	6,8	z_{29}	1,5
II	8	z_{29}	e,n,x:z_{42}
Tamale	8,20	z_{29}	[e,n,z_{15}]
Uno	6,8	z_{29}	[e,n,z_{15}]
II	6,8	z_{29}	e,n,x
Kolda	8,20	z_{35}	1,2
Yarm	6,8	z_{35}	1,2
Angers	8,20	z_{35}	z_6
Apeyeme	8,20	z_{38}	
Diogoye	8,20	z_{41}	z_6
Aesch	6,8	z_{60}	1,2
Group O:9 (D1)			
Sendai	1,9,12	a	1,5
Miami	1,9,12	a	1,5
II	9,12	a	1,5
Os	9,12	a	1,6
Saarbruecken	1,9,12	a	1,7
Lomalinda	1,9,12	a	e,n,x
II	1,9,12	a	e,n,x
Durban	9,12	a	e,n,z_{15}
II	9,12	a	z_{39}
II	1,9,12	a	z_{42}
Onarimon	1,9,12	b	1,2
Frintrop	1,9,12	b	1,5

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
II	1,9,12	b	e,n,x
II	1,9,12	b	z_6
II	1,9,12	b	z_{39}
Goeteborg	9,12	c	1,5
Ipeko	9,12	c	1,6
Elokate	9,12	c	1,7
Alabama	9,12	c	e,n,z_{15}
Ridge	9,12	c	z_6
Ndolo	1,9,12	d	1,5
Tarshyne	9,12	d	1,6
Eschberg	9,12	d	1,7
II	9,12	d	e,n,x
Bangui	9,12	d	e,n,z_{15}
Zega	9,12	d	z_6
Jaffna	1,9,12	d	z_{35}
II	9,12	d	z_{39}
Typhi	9,12[Vi]	d	
Bournemouth	9,12	e,h	1,2
Eastbourne	1,9,12	e,h	1,5
Westafrica	9,12	e,h	1,7
Israel	9,12	e,h	e,n,z_{15}
II	9,12	e,n,x	1,[5],7
II	9,12	e,n,x	1,6
Berta	1,9,12	[f],g,[t]	
Enteritidis	1,9,12	g,m	
Blegdam	9,12	g,m,q	
II	1,9,12	g,m,[s],t	[1,5,7]:[z_{42}]
II	1,9,12	g,m,s,t	e,n,x
Dublin	1,9,12[Vi]	g,p	
Naestved	1,9,12	g,p,s	
Rostock	1,9,12	g,p,u	
Moscow	9,12	g,q	
II	9,12	g,s,t	e,n,x
Newmexico	9,12	g,z_{51}	1,5
II	1,9,12	g,z_{62}	[e,n,x]
Antarctica	9,12	g,z_{63}	
II	9,12	m,t	e,n,x
Pensacola	1,9,12	m,t	[1,2]
II	1,9,12	m,t	1,5
II	1,9,12	m,t	z_{39}
Seremban	9,12	i	1,5
Claibornei	1,9,12	k	1,5
Goverdhan	9,12	k	1,6
Mendoza	9,12	l,v	1,2
Panama	1,9,12	l,v	1,5
Kapemba	9,12	l,v	1,7
Zaiman	9,12	l,v	e,n,x
II	9,12	l,v	e,n,x
Goettingen	9,12	l,v	e,n,z_{15}
II	9,12	l,v	z_{39}
Victoria	1,9,12	l,w	1,5
II	1,9,12	l,w	e,n,x
Itami	9,12	l,z_{13}	1,5
Miyazaki	9,12	l,z_{13}	1,7
Napoli	1,9,12	l,z_{13}	e,n,x
Javiana	1,9,12	l,z_{28}	1,5
Kotu	9,12	l,z_{28}	1,6
II	9,12	l,z_{28}	1,5:[z_{42}]
II	9,12	l,z_{28}	e,n,x
Jamaica	9,12	r	1,5
Camberwell	9,12	r	1,7
Campinense	9,12	r	e,n,z_{15}
Lome	9,12	r	z_6
Powell	9,12	y	1,7
II	1,9,12	y	z_{39}
Mulhouse	1,9,12	z	1,2
Lawndale	1,9,12	z	1,5
Kimpese	9,12	z	1,6

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
II	1,9,12	z	1,7
II	1,9,12	z	z_6
II	9,12	z	z_{39}
Wangata	1,9,12	z_4,z_{23}	[1,7]
Natal	9,12	z_4,z_{24}	
Franken	9,12	z_6	z_{67}
Portland	9,12	z_{10}	1,5
Treguier	9,12	z_{10}	z_6
Ruanda	9,12	z_{10}	e,n,z_{15}
II	9,12	z_{29}	1,5
II	1,9,12	z_{29}	e,n,x
Penarth	9,12	z_{35}	z_6
Elomrane	1,9,12	z_{38}	
II	1,9,12	z_{39}	1,7
Ottawa	1,9,12	z_{41}	1,5
II	1,9,12	z_{42}	1,[5],7
Gallinarum	1,9,12		
Group O:9,46 (D2)			
Baildon	9,46	a	e,n,x
Doba	9,46	a	e,n,z_{15}
Cheltenham	9,46	b	1,5
Zadar	9,46	b	1,6
Worb	9,46	b	e,n,x
II	9,46	b	e,n,x
Bamboye	9,46	b	l,w
Linguere	9,46	b	z_6
Kolar	9,46	b	z_{35}
Itutaba	9,46	c	z_6
Ontario	9,46	d	1,5
Quentin	9,46	d	1,6
Strasbourg	9,46	d	1,7
Olten	9,46	d	e,n,z_{15}
Plymouth	9,46	d	z_6
Bergedorf	9,46	e,h	1,2
Waedenswil	9,46	e,h	1,5
Guerin	9,46	e,h	z_6
II	9,46	e,n,x	1,5,7
Wernigerode	9,46	f,g	
Hillingdon	9,46	g,m	
Macclesfield	9,46	g,m,s	1,2,7
II	9,46	g,[m],[s],t	[e,n,x]
Gateshead	9,46	g,s,t	
II	9,46	g,z_{62}	
II	9,46	m,t	e,n,x
Sangalkam	9,46	m,t	
Mathura	9,46	i	e,n,z_{15}
Potto	9,46	i	z_6
Marylebone	9,46	k	1,2
Cochin	9,46	k	1,5
Ceyco	9,46	k	z_{35}
India	9,46	l,v	1,5
Geraldton	9,46	l,v	1,6
Toronto	9,46	l,v	e,n,x
Ackwepe	9,46	l,w	
Nordrhein	9,46	l,z_{13},z_{28}	e,n,z_{15}
Deckstein	9,46	r	1,7
Shoreditch	9,46	r	e,n,z_{15}
Sokode	9,46	r	z_6
Benin	9,46	y	1,7
Irchel	9,46	y	e,n,x
Nantes	9,46	y	l,w
Mayday	9,46	y	z_6
II	9,46	z	1,5
II	9,46	z	e,n,x
Bambylor	9,46	z	e,n,z_{15}
Ekotedo	9,46	z_4,z_{23}	
II	9,46	z_4,z_{24}	z_{39}:z_{42}
Ngaparou	9,46	z_4,z_{24}	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
Lishabi	9,46	z_{10}	1,7
Inglis	9,46	z_{10}	e,n,x
Mahina	9,46	z_{10}	e,n,z_{15}
Louisiana	9,46	z_{10}	z_6
II	9,46	z_{10}	z_6
II	9,46	z_{10}	z_{39}
Ouakam	9,46	z_{29}	
Hillegersberg	9,46	z_{35}	1,5
Basingstoke	9,46	z_{35}	e,n,z_{15}
Trimdon	9,46	z_{35}	z_6
Fresno	9,46	z_{38}	
II	9,46	z_{39}	1,7
Wuppertal	9,46	z_{41}	
Group O:9,46,27 (D3)			
II	1,9,12,46,27	a	z_6
II	1,9,12,46,27	c	z_{39}
II	9,12,46,27	g,t	e,n,x
II	1,9,12,46,27	l,z_{13},z_{28}	z_{39}
II	1,9,12,46,27	y	z_{39}
II	1,9,12,46,27	z_4,z_{24}	1,5
II	1,9,12,46,27	z_{10}	1,5
II	1,9,12,46,27	z_{10}	e,n,x
II	1,9,12,46,27	z_{10}	z_{39}
Group O:3,10 (E1)[f]			
Aminatu	3,10	a	1,2
Goelzau	3,10[15]	a	1,5
Oxford	3,10[15][15,34]	a	1,7
Masembe	3,10	a	e,n,x
II	3,10	a	e,n,x
Galil	3,10	a	e,n,z_{15}
II	3,10	a	l,v
II	3,10	a	z_{39}
Kalina	3,10	b	1,2
Butantan	3,10[15][15,34]	b	1,5
Allerton	3,10	b	1,6
Huvudsta	3,10	b	1,7
Benfica	3,10	b	e,n,x
II	3,10	b	e,n,x
Yaba	3,10[15]	b	e,n,z_{15}
Epicrates	3,10	b	l,w
Wilmington	3,10	b	z_6
Westminster	3,10[15]	b	z_{35}
II	3,10	b	z_{39}
Asylanta	3,10	c	1,2
Gbadago	3,10[15]	c	1,5
Ikayi	3,10[15]	c	1,6
Pramiso	3,10	c	1,7
Agege	3,10	c	e,n,z_{15}
Anderlecht	3,10	c	l,w
Okefoko	3,10	c	z_6
Stormont	3,10	d	1,2
Shangani	3,10[15]	d	1,5
Lekke	3,10	d	1,6
Onireke	3,10	d	1,7
Souza	3,10[15]	d	e,n,x
II	3,10	d	e,n,x
Madjorio	3,10	d	e,n,z_{15}
Birmingham	3,10[15]	d	l,w
Weybridge	3,10	d	z_6
Maron	3,10	d	z_{35}
Vejle	3,10[15]	e,h	1,2
Muenster	3,10[15][15,34]	e,h	1,5
Anatum	3,10[15][15,34]	e,h	1,6
Nyborg	3,10[15]	e,h	1,7
Newlands	3,10[15,34]	e,h	e,n,x
Lamberhurst	3,10	e,h	e,n,z_{15}
Meleagridis	3,10[15][15,34]	e,h	l,w
Sekondi	3,10	e,h	z_6

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
II	3,10	e,n,x	1,7
Regent	3,10	f,g,[s]	[1,6]
Alfort	3,10	f,g	e,n,x
Suberu	3,10	g,m	
Amsterdam	3,10[15][15,34]	g,m,s	
II	3,10[15]	g,m,s,t	[1,5]
Westhampton	3,10[15][15,34]	g,s,t	
Bloomsbury	3,10	g,t	1,5
II	3,10	g,t	
II	3,10	m,t	1,5
Southbank	3,10[15][15,34]	m,t	[1,6]
II	3,10	m,t	e,n,x
Cuckmere	3,10	i	1,2
Amounderness	3,10	i	1,5
Tibati	3,10	i	1,6
Truro	3,10	i	1,7
Bessi	3,10	i	e,n,x
Falkensee	3,10[15]	i	e,n,z_{15}
Hoboken	3,10	i	l,w
Yeerongpilly	3,10	i	z_6
Wimborne	3,10	k	1,2
Zanzibar	3,10[15]	k	1,5
Serrekunda	3,10	k	1,7
Yundum	3,10	k	e,n,x
Marienthal	3,10	k	e,n,z_{15}
Newrochelle	3,10	k	l,w
Nchanga	3,10[15]	l,v	1,2
Sinstorf	3,10	l,v	1,5
London	3,10[15]	l,v	1,6
Give	3,10[15][15,34]	[d],l,v	1,7
II	3,10	l,v	e,n,x
Ruzizi	3,10	l,v	e,n,z-15
II	3,10	l,v	z_6
Sinchew	3,10	l,v	z_{35}
Assinie	3,10	l,w	z_6
Freiburg	3,10	l,z_{13}	1,2
Uganda	3,10[15]	l,z_{13}	1,5
Fallowfield	3,10	l,z_{13},z_{28}	e,n,z_{15}
Hoghton	3,10	l,z_{13},z_{28}	z_6
II	3,10	l,z_{28}	1,5
Joal	3,10	l,z_{28}	1,7
Lamin	3,10	l,z_{28}	e,n,x
II	3,10	l,z_{28}	e,n,x
II	3,10	l,z_{28}	z_{39}
Ughelli	3,10	r	1,5
Elisabethville	3,10[15]	r	1,7
Simi	3,10	r	e,n,z_{15}
Weltevreden	3,10[15]	r	z_6
Seegefeld	3,10	r,i	1,2
Dumfries	3,10	r,i	1,6
Amager	3,10[15]	y	1,2
Orion	3,10[15][15,34]	y	1,5
Mokola	3,10	y	1,7
Ohlstedt	3,10[15]	y	e,n,x
Bolton	3,10	y	e,n,z_{15}
Langensalza	3,10	y	l,w
Stockholm	3,10[15]	y	z_6
Fufu	3,10	z	1,5
II	3,10	z	1,5
Harleystreet	3,10	z	1,6
Huddinge	3,10	z	1,7
II	3,10	z	e,n,x
Clerkenwell	3,10	z	l,w
Landwasser	3,10	z	z_6
II	3,10	z	z_{39}
Adabraka	3,10	z_4,z_{23}	[1,7]
Wagadugu	3,10	z_4,z_{23}	z_6
Florian	3,10[15]	z_4,z_{24}	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
II	3,10	z_4,z_{24}	
Okerara	3,10	z_{10}	1,2
Lexington	3,10[15][15,34]	z_{10}	1,5
Harrisonburg	3,10[15][15,34]	z_{10}	1,6
Coquilhatville	3,10	z_{10}	1,7
Kristianstad	3,10	z_{10}	e,n,z_{15}
Biafra	3,10	z_{10}	z_6
Everleigh	3,10	z_{29}	e,n,x
II	3,10	z_{29}	[e,n,x]
Jedburgh	3,10[15]	z_{29}	
Zongo	3,10	z_{35}	1,7
Shannon	3,10	z_{35}	l,w
Cairina	3,10	z_{35}	z_6
Macallen	3,10	z_{36}	
Bolombo	3,10	z_{38}	[z_6]
II	3,10	z_{38}	z_{42}
II	3,10	z_{39}	1,[5],7
Pietersburg	3,10[15,34]	z_{69}	1,7
Group O:1,3,19 (E4)			
Niumi	1,3,19	a	1,5
Juba	1,3,19	a	1,7
Gwoza	1,3,19	a	e,n,z_{15}
Alkmaar	1,3,19	a	l,w
Gnesta	1,3,19	b	1,5
Visby	1,3,19	b	1,6
Tambacounda	1,3,19	b	e,n,x
Kande	1,3,19	b	e,n,z_{15}
Broughton	1,3,19	b	l,w
Accra	1,3,19	b	z_6
Eastglam	1,3,19	c	1,5
Bida	1,3,19	c	1,6
Madiago	1,3,19	c	1,7
Ahmadi	1,3,19	d	1,5
Liverpool	1,3,19	d	e,n,z_{15}
Tilburg	1,3,19	d	l,w
Niloese	1,3,19	d	z_6
Vilvoorde	1,3,19	e,h	1,5
Hayindogo	1,3,19	e,h	1,6
Sanktmarx	1,3,19	e,h	1,7
Sao	1,3,19	e,h	e,n,z_{15}
Calabar	1,3,19	e,h	l,w
Rideau	1,3,19	f,g	
Petahtikve	1,3,19	f,g,t	1,7
Maiduguri	1,3,19	f,g,t	e,n,z_{15}
Kouka	1,3,19	g,m,[t]	
Senftenberg	1,3,19	g,[s],t	
Cannstatt	1,3,19	m,t	
Stratford	1,3,19	i	1,2
Chichester	1,3,19	i	1,6
Machaga	1,3,19	i	e,n,x
Avonmouth	1,3,19	i	e,n,z_{15}
Zuilen	1,3,19	i	l,w
Taksony	1,3,19	i	z_6
Oersterbro	1,3,19	k	1,5
Bethune	1,3,19	k	1,7
Ngor	1,3,19	l,v	1,5
Parkroyal	1,3,19	l,v	1,7
Svedvi	1,3,19	l,v	e,n,z_{15}
Fulda	1,3,19	l,w	1,5
Westerstede	1,3,19	l,z_{13}	1,2
Winterthur	1,3,19	l,z_{13}	1,6
Lokstedt	1,3,19	l,z_{13},z_{28}	1,2
Stuivenberg	1,3,19	l,[z_{13}]z_{28}	1,5
Bedford	1,3,19	l,z_{13},z_{28}	e,n,z_{15}
Tomelilla	1,3,19	l,z_{28}	1,7
Kindia	1,3,19	l,z_{28}	e,n,x
Yalding	1,3,19	r	e,n,z_{15}
Fareham	1,3,19	r,i	l,w

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
Gatineau	1,3,19	y	1,5
Thies	1,3,19	y	1,7
Slade	1,3,19	y	e,n,z_{15}
Kinson	1,3,19	y	e,n,x
Krefeld	1,3,19	y	l,w
Korlebu	1,3,19	z	1,5
Kainji	1,3,19	z	1,6
Lerum	1,3,19	z	1,7
Schoeneberg	1,3,19	z	e,n,z_{15}
Carno	1,3,19	z	l,w
Hongkong	1,3,19	z	z_6
Sambre	1,3,19	z_4,z_{24}	
Dallgow	1,3,19	z_{10}	e,n,z_{15}
Llandoff	1,3,19	z_{29}	[z_6]
Ochiogu	1,3,19	z_{38}	[e,n,z_{15}]
Chittagong	1,3,10,19	b	z_{35}
Bilu	1,3,10,19	f,g,t	1,(2),7
Ilugun	1,3,10,19	z_4,z_{23}	z_6
Dessau	1,3,15,19	g,s,t	
Cannonhill	1,3,15,19	y	e,n,x
Group O:11 (F)			
II	11	a	d:e,n,z_{15}
Gallen	11	a	1,2
Marseille	11	a	1,5
VI	11	a	1,5
Toowong	11	a	1,7
Luciana	11	a	e,n,z_{15}
Epinay	11	a	l,z_{13},z_{28}
II	11	a	z_6:z_{42}
Atento	11	b	1,2
Leeuwarden	11	b	1,5
Wohlen	11	b	1,6
VI	11	b	1,7
VI	11	b	e,n,x
Pharr	11	b	e,n,z_{15}
Chiredzi	11	c	1,5
Brindisi	11	c	1,6
II	11	c	e,n,z_{15}
Woodinville	11	c	e,n,x
Ati	11	d	1,2
Gustavia	11	d	1,5
Chandans	11	d	[e,n,x]:[r]
Findorff	11	d	z_6
Chingola	11	e,h	1,2
Adamstua	11	e,h	1,6
Redhill	11	e,h	l,z_{13},z_{28}
Abuja	11	g,m	1,5
Missouri	11	g,s,t	
II	11	g,[m],s,t	z_{39}
IV	11	g,z_{51}	
Moers	11	m,t	
II	11	m,t	e,n,x
Aberdeen	11	i	1,2
Brijbhumi	11	i	1,5
Heerlen	11	i	1,6
Veneziana	11	i	e,n,x
Pretoria	11	k	1,2
Abaetetuba	11	k	1,5
Sharon	11	k	1,6
Colobane	11	k	1,7
Kisarawe	11	k	e,n,x,[z_{15}]
Mannheim	11	k	l,w
Amba	11	k	l,z_{13},z_{28}
IIIb	11	k	z_{53}
Stendal	11	l,v	1,2
Maracaibo	11	l,v	1,5
Fann	11	l,v	e,n,x
Bullbay	11	l,v	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b] Somatic (O) antigen	Flagellar (H) antigen Phase 1	Phase 2
IIIb	11	l,v	z
IIIb	11	l,v	z_{53}
Glidji	11	l,w	1,5
Tours	11	l,z_{13}	1,2
Connecticut	11	l,z_{13},z_{28}	1,5
Osnabrueck	11	l,z_{13},z_{28}	e,n,x
II	11	l,z_{28}	e,n,x
Senegal	11	r	1,5
Rubislaw	11	r	e,n,x
Clanvillian	11	r	e,n,z_{15}
Euston	11	r,i	e,n,x,z_{15}
Volta	11	r	l,z_{13},z_{28}
Solt	11	y	1,5
Jalisco	11	y	1,7
Herzliya	11	y	e,n,x
Crewe	11	z	1,5
Maroua	11	z	1,7
II	11	z	e,n,x
Nyanza	11	z	z_6:[z_{83}]
II	11	z	z_{39}
Remete	11	z_4,z_{23}	1,6
Etterbeek	11	z_4,z_{23}	e,n,z_{15}
IIIa	11	z_4,z_{23}	
IV	11	z_4,z_{23}	
Yehuda	11	z_4,z_{24}	
IV	11	z_4,z_{32}	
Wentworth	11	z_{10}	1,2
Straengnaes	11	z_{10}	1,5
Telhashomer	11	z_{10}	e,n,x
Lene	11	z_{38}	
Maastricht	11	z_{41}	1,2
II	11		1,5
Group O:13 (G)[g]			
Chagoua	1,13,23	a	1,5
II	1,13,23	a	1,5
Mim	13,22	a	1,6
II	13,22	a	e,n,x
Wyldegreen	1,13,23	a	l,w
Marshall	13,22	a	l,z_{13},z_{28}
II	1,13,23	a	z_{42}
Ibadan	13,22	b	1,5
Mississippi	1,13,23	b	1,5
Oudwijk	13,22	b	1,6
II	1,13,23	b	[1,5]:z_{42}
Bracknell	13,23	b	1,6
Rottnest	1,13,22	b	1,7
Vaertan	13,22	b	e,n,x
Ullevi	1,13,23	b	e,n,x
Bahati	13,22	b	e,n,z_{15}
Durham	13,23	b	e,n,z_{15}
Sanktjohann	13,23	b	l,w
II	1,13,22	b	z_{42}
Haouaria	13,22	c	e,n,x,z_{15}
Handen	1,13,23	d	1,2
Mishmarhaemek	1,13,23	d	1,5
Friedenau	13,22	d	1,6
Wichita	1,13,23	d	1,6
Grumpensis	1,13,23	d	1,7
II	13,23	d	e,n,x
Diguel	1,13,22	d	e,n,z_{15}
Telelkebir	13,23	d	e,n,z_{15}
Putten	13,23	d	l,w
Isuge	13,23	d	z_6
Tschangu	1,13,23	e,h	1,5
Willemstad	1,13,22	e,h	1,6
Vridi	1,13,23	e,h	l,w
II	1,13,23	e,n,x	1,[5],7
Raus	13,22	f,g	e,n,x

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Havana	1,13,23	f,g,[s]	
Bron	13,22	g,m	[e,n,z_{15}]
Agbeni	1,13,23	g,m,[s],[t]	
II	1,13,22	g,m,t	[1,5]
II	1,13,23	g,m,s,t	1,5
II	1,13,23	g,m,[s],t	[e,n,x]
II	1,13,23	g,m,s,t	z_{42}
Congo	13,23	g,m,s,t	
Newyork	13,22	g,s,t	
Okatie	13,23	g,[s],t	
II	1,13,22	g,t	1,5
II	1,13,23	g,t	1,5
II	1,13,23	g,[s],t	z_{42}
IIIa	1,13,23	g,z_{51}	
Washington	13,22	m,t	
II	1,13,23	m,t	1,5
II	1,13,23	m,t	e,n,x
II	13,22	m,t	z_{42}:z_{39}
II	1,13,23	m,t	z_{42}
Kintambo	13,23	m,t	
V	1,13,22	i	
Idikan	1,13,23	i	1,5
Jukestown	13,23	i	e,n,z_{15}
Kedougou	1,13,23	i	l,w
II	13,22	k	1,5:z_{42}
Marburg	13,23	k	
II	13,23	k	z_{41}
Lovelace	13,22	l,v	1,5
IIIb	13,22	l,v	1,5,7
Borbeck	13,22	l,v	1,6
Nanga	1,13,23	l,v	e,n,z_{15}
II	13,23	l,w	e,n,x
Taiping	13,22	l,z_{13}	e,n,z_{15}
II	13,22	l,z_{28}	1,5
II	13,23	l,z_{28}	1,5
II	13,23	l,z_{28}	z_6
II	1,13,23	l,z_{28}	z_{42}
V	13,22	r	
Adjame	13,23	r	1,6
Linton	13,23	r	e,n,z_{15}
Tanger	1,13,22	y	1,6
Yarrabah	13,23	y	1,7
Ordonez	1,13,23	y	l,w
Tunis	1,13,23	y	z_6
II	1,13,23	z	1,5
Poona	1,13,22	z	1,6
Farmsen	13,23	z	1,6
Bristol	13,22	z	1,7
Tanzania	1,13,22	z	e,n,z_{15}
Worthington	1,13,23	z	l,w
II	1,13,23	z	z_{42}
II	13,22	z	
Ried	1,13,22	z_4,z_{23}	[e,n,z_{15}]
IIIa	13,22	z_4,z_{23}	
Ajiobo	13,23	z_4,z_{23}	
IIIa	13,23	z_4,z_{23},[z_{32}]	
Romanby	1,13,23	z_4,z_{24}	
IIIa	1,13,23	z_4,z_{24}	
Roodepoort	1,13,22	z_{10}	1,5
II	1,13,22	z_{10}	z_6
Sapele	13,23	z_{10}	e,n,z_{15}
Demerara	13,23	z_{10}	l,w
II	13,22	z_{29}	1,5
II	13,22	z_{29}	e,n,x
II	1,13,23	z_{29}	e,n,x
Agoueve	13,22	z_{29}	
Cubana	1,13,23	z_{29}	
Mampong	13,22	z_{35}	1,6
Nimes	13,22	z_{35}	e,n,z_{15}
Anna	13,23	z_{35}	e,n,z_{15}
Leiden	13,22	z_{38}	
Fanti	13,23	z_{38}	
II	13,22	z_{39}	1,7
II	1,13,23	z_{39}	1,5,7
II	1,13,23	[z_{42}]	1,[5],7
II	13,23		1,6
Group O:6,14 (H)			
Garba	1,6,14,25	a	1,5
VI	[1],6,14	a	1,5
VI	1,6,14,25	a	e,n,x
Banjul	1,6,14,25	a	e,n,z_{15}
Ndjamena	1,6,14,25	b	1,2
Kuntair	1,6,14,25	b	1,5
Tucson	[1],6,14,[25]	b	1,7
IIIb	(6),14	b	e,n,x
Blijdorp	1,6,14,25	c	1,5
Kassberg	1,6,14,25	c	1,6
Runby	1,6,14,25	c	e,n,x
Minna	1,6,14,25	c	l,w
Finkenwerder	[1],6,14,[25]	d	1,5
Woodhull	1,6,14,25	d	1,6
Midway	6,14,24	d	1,7
Florida	[1],6,14,[25]	d	1,7
Lindern	6,14[24]	d	e,n,x
Charity	[1],6,14,[25]	d	e,n,x
Teko	1,6,14,25	d	e,n,z_{15}
Encino	1,6,14,25	d	l,z_{13},z_{28}
Albuquerque	1,6,14,24	d	z_6
Bahrenfeld	6,14,24	e,h	1,5
Onderstepoort	1,6,14,[25]	e,h	1,5
Magumeri	1,6,14,25	e,h	1,6
Beaudesert	[1],6,14,[25]	e,h	1,7
Warragul	[1],6,14,[25]	g,m	
Caracas	[1],6,14,[25]	g,m,s	
Sylvania	[1],6,14,[25]	g,p	
Catanzaro	6,14	g,s,t	
II	1,6,14	m,t	1,5
II	6,14	m,t	e,n,x
Kaitaan	1,6,14,25	m,t	
Mampeza	1,6,14,25	i	1,5
Buzu	[1],6,14,[25]	i	1,7
Schalkwijk	6,14	i	e,n,z_{15}
Moussoro	1,6,14,25	i	e,n,z_{15}
Harburg	[1],6,14,[25]	k	1,5
II	6,14,[24]	k	1,6
II	6,14	k	e,n,x
IIIb	(6),14	k	z
II	1,6,14	k	z_6:z_{42}
IIIb	(6),14	k	z_{53}
Boecker	[1],6,14,[25]	l,v	1,7
Horsham	1,6,14,[25]	l,v	e,n,x
IIIb	(6),14	l,v	z
IIIb	(6),14	l,v	z_{35}
IIIb	(6),14	l,v	z_{53}
Aflao	1,6,14,25	l,z_{28}	e,n,x
Istoria	1,6,14,25	r,i	1,5
IIIb	(6),14	r	z
Surat	[1],6,14,[25]	r,[i]	e,n,z_{15}
Carrau	6,14,[24]	y	1,7
Madelia	1,6,14,25	y	1,7
Fischerkietz	1,6,14,25	y	e,n,x
Mornington	1,6,14,25	y	e,n,z_{15}
Homosassa	1,6,14,25	z	1,5
Kanifing	1,6,14,25	z	1,6
Soahanina	6,14,24	z	e,n,x
Sundsvall	[1],6,14,[25]	z	e,n,x

(*continued*)

TABLE BXII.γ.262. *(cont.)*

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Royan	1,6,14,25	z	e,n,z_{15}
Poano	1,6,14,25	z	l,z_{13},z_{28}
Arapahoe	6,14	z_4,z_{23}	1,5
Bousso	1,6,14,25	z_4,z_{23}	e,n,z_{15}
IV	6,14	z_4,z_{23}	
Chichiri	6,14,24	z_4,z_{24}	
Uzaramo	1,6,14,25	z_4,z_{24}	
Nessa	1,6,14,25	z_{10}	1,2
VI	1,6,14,25	z_{10}	1,(2),7
II	1,6,14	z_{10}	1,5
Laredo	1,6,14,25	z_{10}	1,6
IIIb	(6),14	z_{10}	e,n,x,z_{15}
IIIb	(6),14	z_{10}	z
II	1,6,14	z_{10}	z_6:z_{42}
IIIb	6,14	z_{10}	z_{53}
Potosi	6,14	z_{36}	1,5
Sara	1,6,14,25	z_{38}	e,n,x
II	1,6,14	z_{42}	1,6
IIIb	6,14	z_{52}	e,n,x,z_{15}
IIIb	1,6,14,25	z_{52}	z_{35}
Group 0:16 (I)			
Hannover	16	a	1,2
Brazil	16	a	1,5
Amunigun	16	a	1,6
Nyeko	16	a	1,7
Togba	16	a	e,n,x
Fischerhuette	16	a	e,n,z_{15}
Heron	16	a	z_6
Hull	16	b	1,2
Wa	16	b	1,5
Glasgow	16	b	1,6
Hvittingfoss	16	b	e,n,x
II	16	b	e,n,x
Sangera	16	b	e,n,z_{15}
Vegesack	16	b	l,w
Malstatt	16	b	z_6
II	16	b	z_{39}
II	16	b	z_{42}
Vancouver	16	c	1,5
Gafsa	16	c	1,6
Shamba	16	c	e,n,x
Hithergreen	16	c	e,n,z_{15}
Yoruba	16	c	l,w
Oldenburg	16	d	1,2
Sculcoates	16	d	1,5
II	16	d	1,5
Sherbrooke	16	d	1,6
Gaminara	16	d	1,7
Barranquilla	16	d	e,n,x
Nottingham	16	d	e,n,z_{15}
Caen	16	d	l,w
Barmbek	16	d	z_6
Malakal	16	e,h	1,2
Saboya	16	e,h	1,5
Rhydyfelin	16	e,h	e,n,x
Weston	16	e,h	z_6
II	16	e,n,x	1,(5),7
II	16	e,n,x	1,6:z_{42}
Tees	16	f,g	
Adeoyo	16	g,m,[t]	
Nikolaifleet	16	g,m,s	
II	16	g,[m],[s],t	[1,5]:[z_{42}]
II	16	g,[m],[s],t	[e,n,x]
Cardoner	16	g,s,t	
II	16	m,t	e,n,x
II	16	m,t	[z_{42}]
Mpouto	16	m,t	
Amina	16	i	1,5
Agbara	16	i	1,6
Wisbech	16	i	1,7
Frankfurt	16	i	e,n,z_{15}
Pisa	16	i	l,w
Abobo	16	i	z_6
IIIb	16	i	z_{35}
Szentes	16	k	1,2
Nuatja	16	k	e,n,x
Orientalis	16	k	e,n,z_{15}
IIIb	16	k	z
IIIb	16	(k)	z_{35}
IIIb	16	k	z_{53}
IIIb	16	l,v	1,5,7
Shanghai	16	l,v	1,6
Welikade	16	l,v	1,7
Salford	16	l,v	e,n,x
Burgas	16	l,v	e,n,z_{15}
IIIb	16	l,v	z:[z_{61}]
Losangeles	16	l,v	z_6
IIIb	16	l,v	z_{35}
IIIb	16	l,v	z_{53}
Zigong	16	l,w	1,5
Westeinde	16	l,w	1,6
Brooklyn	16	l,w	e,n,x
Lomnava	16	l,w	e,n,z_{15}
II	16	l,w	z_6
Mandera	16	l,z_{13}	e,n,z_{15}
Enugu	16	l,[z_{13}],z_{28}	[1,5]
Battle	16	l,z_{13},z_{28}	1,6
Ablogame	16	l,z_{13},z_{28}	z_6
II	16	l,z_{28}	z_{42}
Rovaniemi	16	r,i	1,5
Ivory	16	r	1,6
Brunflo	16	r	1,7
Annedal	16	r,i	e,n,x
Zwickau	16	r,i	e,n,z_{15}
Saphra	16	y	1,5
Akuafo	16	y	1,6
Kikoma	16	y	e,n,x
Avignon	16	y	e,n,z_{15}
Gerland	16	z	1,5
Fortlamy	16	z	1,6
Lingwala	16	z	1,7
II	16	z	e,n,x
Brevik	16	z	e,n,[x],z_{15}
Bouake	16	z	z_6
II	16	z	z_{42}
Kibi	16	z_4,z_{23}	[1,6]
II	16	z_4,z_{23}	
IV	16	z_4,z_{23}	
II	16	z_4,z_{24}	
IV	16	z_4,z_{32}	
II	16	z_6	1,6
Badagry	16	z_{10}	1,5
IIIb	16	z_{10}	1,5,7
Lisboa	16	z_{10}	1,6
IIIb	16	z_{10}	e,n,x,z_{15}
Redlands	16	z_{10}	e,n,z_{15}
Angouleme	16	z_{10}	z_6
Saloniki	16	z_{29}	
II	16	z_{29}	1,5
II	16	z_{29}	e,n,x
Trier	16	z_{35}	1,6
Dakota	16	z_{35}	e,n,z_{15}
II	16	z_{35}	e,n,x
IV	16	z_{36}	
II	16	z_{36}	e,n,z_{15}
Naware	16	z_{38}	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Grancanaria	16	z_{39}	[1,6]
II	16	z_{42}	1,(5),7
IIIb	16	z_{52}	z_{35}
Group O:17 (J)			
Bonames	17	a	1,2
Jangwani	17	a	1,5
Kinondoni	17	a	e,n,x
Kirkee	17	b	1,2
Dahra	17	b	1,5
II	17	b	e,n,x,z_{15}
Bignona	17	b	e,n,z_{15}
II	17	b	z_6
Luedinghausen	17	c	1,5
Victoriaborg	17	c	1,6
II	17	c	z_{39}
Berlin	17	d	1,5
Karlshamn	17	d	e,n,z_{15}
Niamey	17	d	l,w
Jubilee	17	e,h	1,2
II	17	e,n,x,z_{15}	1,6
II	17	e,n,x,z_{15}	1,[5],7
II	17	g,m,s,t	
Lowestoft	17	g,s,t	
II	17	g,t	[e,n,x,z_{15}]
II	17	g,t	z_{39}
Bama	17	m,t	
II	17	m,t	
Ahanou	17	i	1,7
IIIb	17	i	z_{35}
Irenea	17	k	1,5
Warri	17	k	1,7
Matadi	17	k	e,n,x
Zaria	17	k	e,n,z_{15}
IIIb	17	k	z
II	17	k	
Morotai	17	l,v	1,2
Michigan	17	l,v	1,5
Lancaster	17	l,v	1,7
Carmel	17	l,v	e,n,x
IIIb	17	l,v	e,n,x,z_{15}
IIIb	17	l,v	z_{35}
Granlo	17	l,z_{28}	e,n,x
Lode	17	r	1,2
IIIb	17	r	z
II	17	y	
Tendeba	17	y	e,n,x
Hadejia	17	y	e,n,z_{15}
Gori	17	z	1,2
Warengo	17	z	1,5
II	17	z	1,7
Tchamba	17	z	e,n,z_{15}
II	17	z	l,w:z_{42}
IIIa	17	z_4,z_{23}	
IIIa	17	z_4,z_{23},z_{32}	
IIIa	17	z_4,z_{24}	
IIIa	17	z_4,z_{32}	
Djibouti	17	z_{10}	e,n,x
IIIb	17	z_{10}	e,n,x,z_{15}
IIIb	17	z_{10}	z
II	17	z_{10}	
Kandla	17	z_{29}	
IIIa	17	z_{29}	
IV	17	z_{29}	
Aachen	17	z_{35}	1,6
IIIa	17	z_{36}	
IV	17	z_{36}	
Group O:18 (K)			
Brazos	6, 14,18	a	e,n,z_{15}
Fluntern	6, 14,18	b	1,5
Rawash	6, 14,18	c	e,n,x
Groenekan	18	d	1,5
Usumbura	18	d	1,7
Pontypridd	18	g,m	
IIIa	18	g,z_{51}	
II	18	m,t	1,5
Langenhorn	18	m,t	
Memphis	18	k	1,5
IIIb	18	(k)	z_{53}
IIIb	18	(k)	z_{54}
IIIb	18	l,v	e,n,x,z_{15}
Orlando	18	l,v	e,n,z_{15}
IIIb	18	l,v	z
IIIb	18	l,v	z_{53}
Toulon	18	l,w	e,n,z_{15}
Tennenlohe	18	r	1,5
IIIb	18	r	z
II	18	y	e,n,x,z_{15}
Potengi	18	z	
Cerro	6, 14,18	z_4,z_{23}	[1,5]
Aarhus	18	z_4,z_{23}	z_{64}
II	18	z_4,z_{23}	
IIIa	18	z_4,z_{23}	
Blukwa	6, 14,18	z_4,z_{24}	
II	18	z_4,z_{24}	
IIIa	18	z_4,z_{32}	
IIIb	18	z_{10}	e,n,x,z_{15}
Leer	18	z_{10}	1,5
Carnac	18	z_{10}	z_6
II	18	z_{10}	z_6
II	18	z_{36}	
IV	18	z_{36},z_{38}	
Sinthia	18	z_{38}	
Delmenhorst	18	z_{71}	
Cotia	18		1,6
Group O:21 (L)			
Assen	21	a	[1,5]
II	21	b	1,5
Ghana	21	b	1,6
Minnesota	21	b	e,n,x
Hydra	21	c	1,6
Rhone	21	c	e,n,x
II	21	c	e,n,x
IIIb	21	c	e,n,x,z_{15}
Spartel	21	d	1,5
Magwa	21	d	e,n,x
Madison	21	d	z_6
Good	21	f,g	e,n,x
II	21	g,[m],[s],t	
IIIa	21	g,z_{51}	
IV	21	g,z_{51}	
II	21	m,t	
Diourbel	21	i	1,2
IIIb	21	i	1,5,7
IIIb	21	i	e,n,x,z_{15}
IIIb	21	k	e,n,x,z_{15}
IIIb	21	k	z
Surrey	21	k	1,2,5
IIIb	21	l,v	z
IIIb	21	l,v	z_{57}
Keve	21	l,w	
Jambur	21	l,z_{28}	e,n,z_{15}
Mountmagnet	21	r	
IIIb	21	r	z
Ibaragi	21	y	1,2
Ruiru	21	y	e,n,x
II	21	z	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Baguida	21	z_4,z_{23}	
IIIa	21	z_4,z_{23}	
IV	21	z_4,z_{23}	
II	21	z_4,z_{24}	
IIIa	21	z_4,z_{24}	
IV	21	z_4,z_{32}	
IIIb	21	z_{10}	e,n,x,z_{15}
IIIb	21	z_{10}	z
II	21	z_{10}	[z_6]
IIIb	21	z_{10}	z_{53}
IIIa	21	z_{29}	
Gambaga	21	z_{35}	e,n,z_{15}
IV	21	z_{36}	
IIIb	21	z_{65}	e,n,x,z_{15}
Group O:28 (M)			
Solna	28	a	1,5
Dakar	28	a	1,6
Bakau	28	a	1,7
Seattle	28	a	e,n,x
II	28	a	e,n,x
Honelis	28	a	e,n,z_{15}
Dibra	28	a	z_6
Moero	28	b	1,5
Ashanti	28	b	1,6
Bokanjac	28	b	1,7
Soumbedioune	28	b	e,n,x
II	28	b	e,n,x
Langford	28	b	e,n,z_{15}
Freefalls	28	b	l,w
II	28	b	z_6
Hermannswerder	28	c	1,5
Eberswalde	28	c	1,6
Halle	28	c	1,7
Dresden	28	c	e,n,x
Wedding	28	c	e,n,z_{15}
Techimani	28	c	z_6
Amoutive	28	d	1,5
Hatfield	28	d	1,6
Mundonobo	28	d	1,7
Mocamedes	28	d	e,n,x
Patience	28	d	e,n,z_{15}
Cullingworth	28	d	l,w
Kpeme	28	e,h	1,7
Gozo	28	e,h	e,n,z_{15}
II	28	e,n,x	1,7
Friedrichsfelde	28	f,g	
Yardley	28	g,m	1,6
Abadina	28	g,m	[e,n,z_{15}]
II	28	g,(m),[s],t	1,5
Croft	28	g,m,s	[e,n,z_{15}]
II	28	g,m,t	e,n,x
II	28	g,m,t	z_{39}
II	28	g,s,t	e,n,x
Ona	28	g,s,t	
II	28	m,t	[e,n,x]
Vinohrady	28	m,t	[e,n,z_{15}]
Morillons	28	m,t	1,6
Doorn	28	i	1,2
Cotham	28	i	1,5
Volkmarsdorf	28	i	1,6
Dieuppeul	28	i	1,7
Warnemuende	28	i	e,n,x
Kuessel	28	i	e,n,z_{15}
Douala	28	i	l,w
Guildford	28	k	1,2
Ilala	28	k	1,5
Adamstown	28	k	1,6
Ikeja	28	k	1,7
Taunton	28	k	e,n,x
Ank	28	k	e,n,z_{15}
Leoben	28	l,v	1,5
Vitkin	28	l,v	e,n,x
Nashua	28	l,v	e,n,z_{15}
Ramsey	28	l,w	1,6
Catalunia	28	l,z_{13},z_{28}	1,5
Penilla	28	l,z_{13},z_{28}	e,n,z_{15}
II	28	l,z_{28}	1,5
Fajara	28	l,z_{28}	e,n,x
Bassadji	28	r	1,6
Kibusi	28	r	e,n,x
II	28	r	e,n,z_{15}
Fairfield	28	r	l,w
Chicago	28	r,[i]	1,5
Banco	28	r,i	1,7
Sanktgeorg	28	r,[i]	e,n,z_{15}
Oskarshamn	28	y	1,2
Nima	28	y	1,5
Pomona	28	y	1,7:[z_{60}]
Kitenge	28	y	e,n,x
Telaviv	28	y	e,n,z_{15}
Shomolu	28	y	l,w
Selby	28	y	z_6
Vanier	28	z	1,5
II	28	z	1,5
Doel	28	z	1,6
Ezra	28	z	1,7
Brisbane	28	z	e,n,z_{15}
II	28	z	z_{39}
Cannobio	28	z_4,z_{23}	1,5
Teltow	28	z_4,z_{23}	1,6
Babelsberg	28	z_4,z_{23}	[e,n,z_{15}]
Rogy	28	z_{10}	1,2
Farakan	28	z_{10}	1,5
Libreville	28	z_{10}	1,6
Malaysia	28	z_{10}	1,7
Umbilo	28	z_{10}	e,n,x
Luckenwalde	28	z_{10}	e,n,z_{15}
Moroto	28	z_{10}	l,w
IIIb	28	z_{10}	z
Djermaia	28	z_{29}	
II	28	z_{29}	1,5
II	28	z_{29}	e,n,x
Konolfingen	28	z_{35}	1,6
Balili	28	z_{35}	1,7
Santander	28	z_{35}	e,n,z_{15}
Aderike	28	z_{38}	e,n,z_{15}
Group O:30 (N)			
Overvecht	30	a	1,2
Zehlendorf	30	a	1,5
Guarapiranga	30	a	e,n,x
Doulassame	30	a	e,n,z_{15}
II	30	a	z_{39}
Louga	30	b	1,2
Aschersleben	30	b	1,5
Urbana	30	b	e,n,x
Neudorf	30	b	e,n,z_{15}
II	30	b	z_6
Zaire	30	c	1,7
Morningside	30	c	e,n,z_{15}
II	30	c	z_{39}
Messina	30	d	1,5
Livulu	30	e,h	1,2
Torhout	30	e,h	1,5
Godesberg	30	g,m,[t]	
II	30	g,m,s	e,n,x
Giessen	30	g,m,s	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Sternschanze	30	g,s,t	
II	30	g,t	
Wayne	30	g,z_{51}	
II	30	m,t	
Landau	30	i	1,2
Morehead	30	i	1,5
Mjordan	30	i	e,n,z_{15}
Soerenga	30	i	l,w
Hilversum	30	k	1,2
Ramatgan	30	k	1,5
Aqua	30	k	1,6
Angoda	30	k	e,n,x
Odozi	30	k	e,n,[x],z_{15}
II	30	k	e,n,x,z_{15}
Ligeo	30	l,v	1,2
Donna	30	l,v	1,5
Ockenheim	30	l,z_{13},z_{28}	1,6
Morocco	30	l,z_{13},z_{28}	e,n,z_{15}
II	30	l,z_{28}	z_6
Grandhaven	30	r	1,2
Gege	30	r	1,5
Matopeni	30	y	1,2
Bietri	30	y	1,5
Steinplatz	30	y	1,6
Baguirmi	30	y	e,n,x
Nijmegen	30	y	e,n,z_{15}
Stoneferry	30	z_4,z_{23}	
Bodjonegoro	30	z_4,z_{24}	
II	30	z_6	1,6
Sada	30	z_{10}	1,2
Senneville	30	z_{10}	1,5
Kumasi	30	z_{10}	e,n,z_{15}
II	30	z_{10}	e,n,x,z_{15}
Aragua	30	z_{29}	
Kokoli	30	z_{35}	1,6
Wuiti	30	z_{35}	e,n,z_{15}
Ago	30	z_{38}	
II	30	z_{39}	1,7
Group O:35 (O)			
Umhlatazana	35	a	e,n,z_{15}
Tchad	35	b	
Gouloumbo	35	c	1,5
Yolo	35	c	[e,n,z_{15}]
II	35	d	1,5
Dembe	35	d	l,w
Gassi	35	e,h	z_6
Adelaide	35	f,g	
Ealing	35	g,m,s	
II	35	g,m,s,t	
Ebrie	35	g,m,t	
Anecho	35	g,s,t	
II	35	g,t	1,5
II	35	g,t	z_{42}
Agodi	35	g,t	
IIIa	35	g,z_{51}	
Monschaui	35	m,t	
II	35	m,t	
IIIb	35	i	e,n,x,z_{15}
Gambia	35	i	e,n,z_{15}
Bandia	35	i	l,w
IIIb	35	i	z
IIIb	35	i	z_{35}
IIIb	35	i	z_{53}
IIIb	35	k	e,n,x,z_{15}
IIIb	35	k	z
IIIb	35	(k)	z
IIIb	35	(k)	z_{35}
IIIb	35	k	z_{53}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IIIb	35	l,v	1,5,7
IIIb	35	l,v	z_{35}:[z_{67}]
II	35	l,z_{28}	
IIIb	35	r	e,n,x,z_{15}
Massakory	35	r	l,w
IIIb	35	r	z
IIIb	35	r	z_{35}
IIIb	35	r	z_{61}
Alachua	35	z_4,z_{23}	
IIIa	35	z_4,z_{23}	
Westphalia	35	z_4,z_{24}	
IIIa	35	z_4,z_{32}	
Camberene	35	z_{10}	1,5
Enschede	35	z_{10}	l,w
Ligna	35	z_{10}	z_6
IIIb	35	z_{10}	z_{35}
II	35	z_{29}	e,n,x
Widemarsh	35	z_{29}	
IIIa	35	z_{29}	
IIIa	35	z_{36}	
Haga	35	z_{38}	
IIIb	35	z_{52}	1,5,7
IIIb	35	z_{52}	e,n,x,z_{15}
IIIb	35	z_{52}	z
IIIb	35	z_{52}	z_{35}
Group O:38 (P)			
Oran	38	a	e,n,z_{15}
II	38	b	1,2
Rittersbach	38	b	e,n,z_{15}
Sheffield	38	c	1,5
Kidderminster	38	c	1,6
II	38	d	[1,5]
Thiaroye	38	e,h	1,2
Kasenyi	38	e,h	1,5
Korovi	38	g,m,[s]	
II	38	g,t	
IIIa	38	g,z_{51}	
IV	38	g,z_{51}	
Rothenburgsort	38	m,t	
Mgulani	38	i	1,2
Lansing	38	i	1,5
IIIb	38	i	z
IIIb	38	i	z_{53}
Echa	38	k	1,2
Mango	38	k	1,5
Inverness	38	k	1,6
Njala	38	k	e,n,x
IIIb	38	k	e,n,x,z_{15}
IIIb	38	k	z
IIIb	38	k	z_{53}
IIIb	38	(k)	1,5,7
IIIb	38	(k)	z
IIIb	38	(k)	z_{35}
IIIb	38	(k)	z_{54}
IIIb	38	(k)	z_{54}
Alger	38	l,v	1,2
Kimberley	38	l,v	1,5
Taylor	38	l,v	e,n,z_{15}
Roan	38	l,v	e,n,x
IIIb	38	l,v	z
IIIb	38	l,v	z_{35}
IIIb	38	l,v	z_{53}:[z_{54}]
Lindi	38	r	1,5
IIIb	38	r	1,5,7
Emmastad	38	r	1,6
IIIb	38	r	e,n,x,z_{15}
IIIb	38	r	z:[z_{57}]
IIIb	38	r	z_{35}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Freetown	38	y	1,5
Colombo	38	y	1,6
Perth	38	y	e,n,x
Stachus	38	z	
Yoff	38	z_4,z_{23}	1,2
IIIa	38	z_4,z_{23}	
IV	38	z_4,z_{23}	
Bangkok	38	z_4,z_{24}	
Neunkirchen	38	z_{10}	
IIIb	38	z_{10}	z
IIIb	38	z_{10}	z_{53}
Klouto	38	z_{38}	
IIIb	38	z_{52}	z_{35}
IIIb	38	z_{52}	z_{53}
IIIb	38	z_{53}	
IIIb	38	z_{61}	[z_{53}]
Group O:39 (Q)			
II	39	a	z_{39}
Wandsworth	39	b	1,2
Abidjan	39	b	l,w
II	39	c	e,n,x
Logone	39	d	1,5
Mara	39	e,h	1,5
II	39	e,n,x	1,7
II	39	[g],m,t	[e,n,x]
Hofit	39	i	1,5
Cumberland	39	i	e,n,x
Alma	39	i	e,n,z_{15}
Champaign	39	k	1,5
II	39	l,v	1,5
Kokomlemle	39	l,v	e,n,x
Oerlikon	39	l,v	e,n,z_{15}
II	39	l,z_{28}	e,n,x
II	39	l,z_{28}	z_{39}
Anfo	39	y	1,2
Windermere	39	y	1,5
Hegau	39	z_{10}	
II	39		1,7
Group O:40 (R)			
Shikmonah	40	a	1,5
Greiz	40	a	z_6
II	1,40	a	z_6
II	40	a	z_{39}
Riogrande	40	b	1,5
Saugus	40	b	1,7
Johannesburg	1,40	b	e,n,x
Duval	1,40	b	e,n,z_{15}
Benguella	40	b	z_6
II	40	b	
II	1,40	c	e,n,x,z_{15}
II	1,40	c	z_{39}
Driffield	1,40	d	1,5
II	40	d	
Tilene	1,40	e,h	1,2
II	1,40	e,n,x	1,[5],7
II	1,40	e,n,x,z_{15}	1,6
Bijlmer	1,40	g,m	
II	1,40	g,[m],[s],[t]	e,n,x
II	1,40	g,[m],[s],t	1,5
II	1,40	g,t	e,n,x,z_{15}
II	40	g,t	z_{39}
IV	1,40	g,t	
II	1,40	g,[m],[s],t	z_{42}
IIIa	40	g,z_{51}	
IIIb	40	g,z_{51}	e,n,x,z_{15}
IV	1,40	g,z_{51}	
II	40	m,t	z_{39}
II	1,40	m,t	z_{42}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IV	40	m,t	
IIIb	40	i	1,5,7
Goulfey	1,40	k	1,5
Allandale	1,40	k	1,6
Hann	40	k	e,n,x
II	1,40	k	e,n,x,z_{15}
IIIb	40	k	z:z_{57}
II	40	k	z_6
IIIb	40	k	z_{53}
Millesi	1,40	l,v	1,2
Canary	40	l,v	1,6
II	40	l,v	e,n,x
IIIb	40	l,v	z
IIIb	40	l,v	z_{53}
Overchurch	1,40	l,w	[1,2]
Tiko	1,40	l,z_{13},z_{28}	1,2
Bukavu	1,40	l,z_{28}	1,5
II	1,40	l,z_{28}	1,5:z_{42}
Santhiaba	40	l,z_{28}	1,6
II	1,40	l,z_{28}	z_{39}
Odienne	40	y	1,5
II	1,40	z	1,5
Casamance	40	z	e,n,x
Nowawes	40	z	z_6
II	1,40	z	z_6
II	1,40	z	z_{39}
II	40	z	z_{42}
IIIa	40	z_4,z_{23}	
IV	1,40	z_4,z_{23}	
II	40	z_4,z_{24}	z_{39}
IIIa	40	z_4,z_{24}	
IV	40	z_4,z_{24}	
IIIa	40	z_4,z_{32}	
IV	40	z_4,z_{32}	
II	1,40	z_6	1,5
Trotha	40	z_{10}	z_6
IIIb	40	z_{10}	z_{35}
Omifisan	1,40	z_{29}	
IIIa	40	z_{29}	
II	1,40	z_{35}	e,n,x,z_{15}
Yekepa	1,40	z_{35}	e,n,z_{15}
V	1,40	z_{35}	
IIIa	40	z_{36}	
II	1,40	z_{39}	1,5:z_{42}
II	1,40	z_{39}	1,6
IIIb	40	z_{39}	1,6
II	40	z_{39}	1,7
Karamoja	1,40	z_{41}	1,2
II	1,40	z_{42}	1,6
II	1,40	[z_{42}]	1,(5),7
V	1,40	z_{81}	
Group O:41 (S)			
Burundi	41	a	
II	41	b	1,5
Vaugirard	41	b	1,6
VI	41	b	1,7
Vietnam	41	b	z_6
Sica	41	b	e,n,z_{15}
IIIb	41	c	e,n,x,z_{15}
II	41	c	z_6
Egusi	41	d	1,5
II	41	d	z_6
II	41	g,m,s,t	z_6
II	41	g,t	
IIIa	41	g,z_{51}	
Leatherhead	41	m,t	1,6
Samaru	41	i	1,5
Verona	41	i	1,6

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Ferlo	41	k	1,6
II	41	k	1,6
II	41	k	z_6
IIIb	41	(k)	z_{35}
II	41	l,z_{13},z_{28}	e,n,x,z_{15}
Lubumbashi	41	r	1,5
II	41	z	1,5
Bofflens	41	z_4,z_{23}	1,7
Waycross	41	z_4,z_{23}	[e,n,z_{15}]
IIIa	41	z_4,z_{23}	
IV	41	z_4,z_{23}	
IIIa	41	z_4,z_{23},z_{32}	
Ipswich	41	z_4,z_{24}	1,5
IIIa	41	z_4,z_{24}	
IIIa	41	z_4,z_{32}	
II	41	z_{10}	1,2
Leipzig	41	z_{10}	1,5
Landala	41	z_{10}	1,6
Inpraw	41	z_{10}	e,n,x
II	41	z_{10}	e,n,x,z_{15}
II	41	z_{10}	z_6
Lodz	41	z_{29}	
IIIa	41	z_{29}	
IV	41	z_{29}	
Ahoutoue	41	z_{35}	1,6
IIIa	41	z_{36}	
Offa	41	z_{38}	
IV	41	z_{52}	
II	41		1,6
Group O:42 (T)			
Faji	1,42	a	e,n,z_{15}
II	42	b	1,5
Orbe	42	b	1,6
II	42	b	e,n,x,z_{15}
Tomegbe	1,42	b	e,n,z_{15}
Egusitoo	1,42	b	z_6
II	42	b	z_6
Antwerpen	1,42	c	e,n,z_{15}
Kampala	1,42	c	z_6
II	42	d	z_6
II	42	e,n,x	1,6
II	42	g,t	
Maricopa	1,42	g,z_{51}	1,5
IIIa	42	g,z_{51}	
IV	1,42	g,z_{51}	
II	42	m,t	[e,n,x,z_{15}]
Waral	1,42	m,t	
Kaneshie	1,42	i	l,w
Borromea	42	i	1,6
Middlesbrough	1,42	i	z_6
Haferbreite	42	k	1,6
IIIb	42	k	e,n,x,z_{15}
IIIb	42	k	z
Gwale	1,42	k	z_6
IIIb	42	(k)	z_{35}
IIIb	42	l,v	1,5,7
II	42	l,v	e,n,x,z_{15}
IIIb	42	l,v	e,n,x,z_{15}
Coogee	42	l,v	e,n,z_{15}
IIIb	42	l,v	z
IIIb	42	l,v	z_{53}
II	1,42	l,w	e,n,x
II	1,42	l,[z_{13}],z_{28}	z_6
Sipane	1,42	r	e,n,z_{15}
Brive	1,42	r	l,w
IIIb	42	r	z
IIIb	42	r	z_{53}
II	42	r	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IIIa	42	r	
Spalentor	1,42	y	e,n,z_{15}
Harvestehude	1,42	y	z_6
II	42	z	1,5
Ursenbach	1,42	z	1,6
II	42	z	e,n,x,z_{15}
Melbourne	42	z	e,n,z_{15}
II	42	z	z_6
Gera	1,42	z_4,z_{23}	1,6
Broc	42	z_4,z_{23}	e,n,z_{15}
IIIa	42	z_4,z_{23}	
Toricada	1,42	z_4,z_{24}	
IIIa	42	z_4,z_{24}	
IV	1,42	z_4,z_{24}	
II	42	z_6	1,6
II	42	z_{10}	1,2
II	42	z_{10}	e,n,x,z_{15}
IIIb	42	z_{10}	e,n,x,z_{15}
IIIb	42	z_{10}	z
Loenga	1,42	z_{10}	z_6
II	42	z_{10}	z_6
IIIb	42	z_{10}	z_{35}
IIIb	42	z_{10}	z_{67}
Djama	1,42	z_{29}	[1,5]
Kahla	1,42	z_{35}	1,6
Hennekamp	42	z_{35}	e,n,z_{15}
Tema	1,42	z_{35}	z_6
Weslaco	42	z_{36}	
IV	42	z_{36}	
Vogan	1,42	z_{38}	z_6
Taset	1,42	z_{41}	
IIIb	42	z_{52}	z
Group O:43 (U)			
Graz	43	a	1,2
Berkeley	43	a	1,5
II	43	a	1,5
II	43	a	z_6
Niederoderwitz	43	b	
II	43	b	z_{42}
Montreal	43	c	1,5
Orleans	43	d	1,5
II	43	d	e,n,x,z_{15}
II	43	d	z_{39}
II	43	d	z_{42}
II	43	e,n,x,z_{15}	1,(5),7
II	43	e,n,x,z_{15}	1,6
Milwaukee	43	f,g,[t]	
II	43	g,m,[s],t	[z_{42}]
II	43	g,t	[1,5]
IIIa	43	g,z_{51}	
IV	43	g,z_{51}	
II	43	g,z_{62}	e,n,x
Mbao	43	i	1,2
Voulte	43	i	e,n,x
Thetford	43	k	1,2
Ahuza	43	k	1,5
IIIb	43	k	z
IIIb	43	l,v	z_{53}
Sudan	43	l,z_{13}	
II	43	l,z_{13},z_{28}	1,5
IIIb	43	r	e,n,x,z_{15}
IIIb	43	r	z
IIIb	43	r	z_{53}
Farcha	43	y	1,2
Kingabwa	43	y	1,5
Ogbete	43	z	1,5
II	43	z	1,5
Arusha	43	z	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
II	43	z_4,z_{23}	
IIIa	43	z_4,z_{23}	
IV	43	z_4,z_{23}	
IIIa	43	z_4,z_{24}	
IV	43	z_4,z_{24}	
IV	43	z_4,z_{32}	
Adana	43	z_{10}	1,5
II	43	z_{29}	e,n,x
II	43	z_{29}	z_{42}
Makiling	43	z_{29}	
IV	43	z_{29}	
Ahepe	43	z_{35}	1,6
IIIa	43	z_{36}	
IV	43	z_{36},z_{38}	
Irigny	43	z_{38}	
II	43	z_{42}	1,5,7
IIIb	43	z_{52}	z_{53}
Group O:44 (V)			
IV	44	a	
Niakhar	44	a	1,5
Tiergarten	44	a	e,n,x
Niarembe	44	a	l,w
Sedgwick	44	b	e,n,z_{15}
Madigan	44	c	1,5
Quebec	44	c	e,n,z_{15}
Bobo	44	d	1,5
Kermel	44	d	e,n,x
Fischerstrasse	44	d	e,n,z_{15}
Palamaner	1,44	d	z_{35}
II	1,44	e,n,x	1,6
Vleuten	44	f,g	
Gamaba	1,44	g,m,[s]	
Splott	44	g,s,t	
II	44	g,t	z_{42}
IIIb	44	g,t	$1,5:z_{42}$
Carswell	44	g,z_{51}	
IV	44	g,z_{51}	-
Muguga	44	m,t	
Maritzburg	1,44	i	e,n,z_{15}
Lawra	44	k	e,n,z_{15}
Malika	44	l,z_{28}	1,5
Brefet	44	r	e,n,z_{15}
V	44	r	
Uhlenhorst	44	z	l,w
Bolama	44	z	e,n,x
Kua	44	z_4,z_{23}	
Ploufragan	1,44	z_4,z_{23}	e,n,z_{15}
II	44	z_4,z_{23}	
IIIa	44	z_4,z_{23}	
IV	44	z_4,z_{23}	
IIIa	44	z_4,z_{23},z_{32}	
Christiansborg	44	z_4,z_{24}	
IIIa	44	z_4,z_{24}	
IV	44	z_4,z_{24}	
IIIa	44	z_4,z_{32}	
IV	1,44	z_4,z_{32}	
Guinea	1,44	z_{10}	1,7
Llobregat	44	z_{10}	e,n,x
II	44	z_{29}	$e,n,x:z_{42}$
Zinder	44	z_{29}	
IV	44	z_{29}	
IV	44	$z_{36},[z_{38}]$	
Koketime	44	z_{38}	
II	1,44	z_{39}	e,n,x,z_{15}
V	44	z_{39}	
Group O:45 (W)			
VI	45	a	e,n,x
Meekatharra	45	a	e,n,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
II	45	a	z_{10}
Riverside	45	b	1,5
Fomeco	45	b	e,n,z_{15}
Deversoir	45	c	e,n,x
Dugbe	45	d	1,6
Karachi	45	d	e,n,x
Warmsen	45	d	e,n,z_{15}
Suelldorf	45	f,g	
Tornow	45	g,m,[s],[t]	
II	45	g,m,s,t	1,5
II	45	g,m,s,t	e,n,x
II	45	g,m,t	e,n,x,z_{15}
Binningen	45	g,s,t	
IIIa	45	g,z_{51}	
IV	45	g,z_{51}	
II	45	m,t	1,5
Apapa	45	m,t	
Verviers	45	k	1,5
Casablanca	45	k	1,7
Cairns	45	k	e,n,z_{15}
Imo	45	l,v	$[e,n,z_{15}]$
Kofandoka	45	r	e,n,z_{15}
II	45	z	1,5
Yopougon	45	z	e,n,z_{15}
II	45	z	z_{39}
IIIa	45	z_4,z_{23}	
IV	45	z_4,z_{23}	
Transvaal	45	z_4,z_{24}	
IIIa	45	z_4,z_{24}	
IIIa	45	z_4,z_{32}	
Aprad	45	z_{10}	
Jodhpur	45	z_{29}	
II	45	z_{29}	1,5
II	45	z_{29}	e,n,x
II	45	z_{29}	z_{42}
IIIa	45	z_{29}	–
Lattenkamp	45	z_{35}	1,5
Balcones	45	z_{36}	
IV	45	z_{36},z_3	
Group O:47 (X)			
II	47	a	1,5
II	47	a	e,n,x,z_{15}
Wenatchee	47	b	1,2
II	47	b	1,5
II	47	b	e,n,x,z_{15}
Sya	47	b	z_6
II	47	b	z_6
IIIb	47	c	1,5,7
Kodjovi	47	c	1,6
IIIb	47	c	$e,n,x,z_{15}:[z_{57}]$
IIIb	47	c	z
IIIb	47	c	z_{35}
Stellingen	47	d	e,n,x
II	47	d	z_{39}
II	47	e,n,x,z_{15}	1,6
Sljeme	1,47	f,g	
Luke	1,47	g,m	
II	47	g,t	e,n,x
IIIa	47	g,z_{51}	
Mesbit	47	m,t	e,n,z_{15}
IIIb	47	i	e,n,x,z_{15}
Bergen	47	i	e,n,z_{15}
IIIb	47	i	z
IIIb	47	i	z_{35}
IIIb	47	i	$z_{53}:[z_{57}]$
Staoueli	47	k	1,2
Bootle	47	k	1,5
IIIb	47	k	1,5,7

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Dahomey	47	k	1,6
IIIb	47	k	e,n,x,z_{15}
Lyon	47	k	e,n,z_{15}
IIIb	47	k	z
IIIb	47	k	z_{35}
IIIb	47	k	z_{53}
IIIb	47	l,v	1,5,(7)
IIIb	47	l,v	e,n,x,z_{15}
IIIb	47	l,v	z
IIIb	47	l,v	z_{35}
IIIb	47	l,v	z_{53}
IIIb	47	l,v	z_{57}
IV	47	l,v	
Teshie	1,47	l,z_{13},z_{28}	e,n,z_{15}
IIIb	47	r	e,n,x,z_{15}
Dapango	47	r	1,2
IIIb	47	r	1,5,7
IIIb	47	r	z
IIIb	47	r	z_{35}
IIIb	47	r	z_{53}:[z_{60}]
IIIa	47	r	
Moualine	47	y	1,6
Blitta	47	y	e,n,x
Mountpleasant	47	z	1,5
Kaolack	47	z	1,6
II	47	z	e,n,x,z_{15}
II	47	z	z_6
Tabligbo	47	z_4,z_{23}	e,n,z_{15}
Binche	47	z_4,z_{23}	l,w
Bere	47	z_4,z_{23}	z_6
IIIa	47	z_4,z_{23}	
Tamberma	47	z_4,z_{24}	
II	47	z_6	1,6
IIIb	47	z_{10}	1,5,7
Namoda	47	z_{10}	e,n,z_{15}
IIIb	47	z_{10}	z
IIIb	47	z_{10}	z_{35}
II	47	z_{29}	e,n,x,z_{15}
Ekpoui	47	z_{29}	
IIIa	47	z_{29}	
Bingerville	47	z_{35}	e,n,z_{15}
IV	47	z_{36}	
Alexanderplatz	47	z_{38}	
Quinhon	47	z_{44}	
IIIb	47	z_{52}	1,5,7
IIIb	47	z_{52}	e,n,x,z_{15}
IIIb	47	z_{52}	z
IIIb	47	z_{52}	z_{35}
Group O:48 (Y)			
Hisingen	48	a	1,5,7
II	48	a	z_6
II	48	a	z_{39}
II	48	b	z_6
V	48	b	
IIIb	48	c	z
II	48	d	1,2
II	48	d	z_6
Buckeye	48	d	
Fitzroy	48	e,h	1,5
II	48	e,n,x,z_{15}	z_6
II	48	g,m,t	
IIIa	48	g,z_{51}	
IV	48	g,z_{51}	
IIIb	48	i	z
IIIb	48	i	z_{35}:[z_{57}]
IIIb	48	i	z_{53}
IIIb	48	i	z_{61}
V	48	i	

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IIIb	48	k	1,5,(7)
II	48	k	e,n,x,z_{15}
IIIb	48	k	e,n,x,z_{15}
Dahlem	48	k	e,n,z_{15}
IIIb	48	k	z
IIIb	48	k	z_{35}
II	48	k	z_{39}
IIIb	48	k	z_{53}
IIIb	48	(k)	z_{53}
Australia	48	l,v	1,5
IIIb	48	l,v	1,5,(7)
IIIb	48	l,v	z
IIIb	48	r	e,n,x,z_{15}
IIIb	48	r	z
Toucra	48	z	1,5
II	48	z	1,5
IIIb	48	z	1,5,7
IIIa	48	z_4,z_{23}	
IV	48	z_4,z_{23}	
IIIa	48	z_4,z_{23},z_{32}	
Djakarta	48	z_4,z_{24}	
IIIa	48	z_4,z_{24}	
IIIa	48	z_4,z_{32}	
IV	48	z_4,z_{32}	
II	48	z_{10}	[1,5]
VI	48	z_{10}	1,5
Isaszeg	48	z_{10}	e,n,x
IIIb	48	z_{10}	e,n,x,z_{15}
IIIb	48	z_{10}	z
II	48	z_{29}	
IV	48	z_{29}	
IIIb	48	z_{35}	z_{52}
V	48	z_{35}	
IIIa	48	z_{36}	
IV	48	z_{36},[z_{38}]	
V	48	z_{39}	
V	48	z_{41}	
IIIb	48	z_{52}	e,n,x,z_{15}
IIIb	48	z_{52}	z
V	48	z_{65}	
V	48	z_{81}	
Group O:50 (Z)			
IV	50	a	
Rochdale	50	b	e,n,x
II	50	b	z_6
IV	50	b	
Hemingford	50	d	1,5
IV	50	d	
II	50	e,n,x	1,7
II	50	g,[m],s,t	[1,5]
IV	50	g,z_{51}	
II	50	g,z_{62}	e,n,x
II	50	m,t	z_6:z_{42}
IIIb	50	i	1,5,7
IIIb	50	i	e,n,x,z_{15}
IIIb	50	i	z
IIIb	50	k	1,5,7
II	50	k	e,n,x:z_{42}
IIIb	50	k	e,n,x,z_{15}
IIIb	50	k	z
IIIb	50	(k)	z
II	50	k	z_6
IIIb	50	k	z_{35}
IIIb	50	(k)	z_{35}
IIIb	50	k	z_{53}
Fass	50	l,v	1,2
IIIb	50	l,v	e,n,x,z_{15}
IIIb	50	l,v	z

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IIIb	50	l,v	z_{35}
VI	50	l,v	z_{67}
II	50	l,w	e,n,x,z_{15}:z_{42}
II	50	l,z_{28}	z_{42}
IIIb	50	r	1,5,(7)
IIIb	50	r	e,n,x,z_{15}
IIIb	50	r	z
IIIb	50	r	z_{35}
IIIb	50	r	z_{53}
Dougi	50	y	1,6
II	50	z	e,n,x
IIIb	50	z	z_{52}
IIIa	50	z_4,z_{23}	
IV	50	z_4,z_{23}	
IIIa	50	z_4,z_{23},z_{32}	
IIIa	50	z_4,z_{24}	
IV	50	z_4,z_{24}	
IIIa	50	z_4,z_{32}	
IV	50	z_4,z_{32}	
IIIb	50	z_{10}	z
II	50	z_{10}	z_6:z_{42}
IIIb	50	z_{10}	z_{53}
Ivorycoast	50	z_{29}	
IIIa	50	z_{29}	
IIIa	50	z_{36}	
II	50	z_{42}	1,7
IIIb	50	z_{52}	1,5,7
IIIb	50	z_{52}	z_{35}
IIIb	50	z_{52}	z_{53}
Group O:51			
IV	51	a	
Tione	51	a	e,n,x
Karaya	51	b	1,5
IV	51	b	
II	51	c	
Gokul	1,51	d	1,5
Meskin	51	e,h	1,2
II	51	g,s,t	e,n,x
IIIa	51	g,z_{51}	
Kabete	51	i	1,5
Dan	51	k	e,n,z_{15}
IIIb	51	k	z_{35}
Harcourt	51	l,v	1,2
Overschie	51	l,v	1,5
Dadzie	51	l,v	e,n,x
IIIb	51	l,v	z
Moundou	51	l,z_{28}	1,5
II	51	l,z_{28}	z_6
II	51	l,z_{28}	z_{39}
Lutetia	51	r,i	l,z_{13},z_{28}
Antsalova	51	z	1,5
Treforest	1,51	z	1,6
Lechler	51	z	e,n,z_{15}
IIIa	51	z_4,z_{23}	
IV	51	z_4,z_{23}	
IIIa	51	z_4,z_{24}	
IIIa	51	z_4,z_{32}	
Bergues	51	z_{10}	1,5
II	51	z_{29}	e,n,x,z_{15}
II	51		1,7
Group O:52			
Uithof	52	a	1,5
Ord	52	a	e,n,z_{15}
Molesey	52	b	1,5
Flottbek	52	b	e,n,x
II	52	c	k
Utrecht	52	d	1,5
II	52	d	e,n,x,z_{15}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
II	52	d	z_{39}
Butare	52	e,h	1,6
Derkle	52	e,h	1,7
Saintemarie	52	g,t	
II	52	g,t	
Bordeaux	52	k	1,5
IIIb	52	k	z_{35}
IIIb	52	(k)	z_{35}
IIIb	52	k	z_{53}
IIIb	52	l,v	z_{53}
II	52	z	z_{39}
IIIb	52	z	z_{52}
II	52	z_{39}	1,5,7
II	52	z_{44}	1,5,7
Group O:53			
II	53	c	1,5
II	53	d	1,5
II	1,53	d	z_{39}
II	53	d	z_{42}
IIIa	53	g,z_{51}	
IV	1,53	g,z_{51}	
IIIb	53	i	z
IIIb	53	k	e,n,x,z_{15}
IIIb	53	k	z
IIIb	53	(k)	z
IIIb	53	(k)	z_{35}
IIIb	53	k	z_{53}
IIIb	53	l,v	e,n,x,z_{15}
IIIb	53	l,v	z
IIIb	53	l,v	z_{35}
II	53	l,z_{28}	e,n,x
II	53	l,z_{28}	z_6
II	53	l,z_{28}	z_{39}
IIIb	53	r	z
IIIb	53	r	z_{35}
IIIb	53	r	z_{68}
II	53	z	1,5
IIIb	53	z	1,5,(7)
II	53	z	z_6
IIIa	53	z_4,z_{23}	
IV	53	z_4,z_{23}	
IIIa	53	z_4,z_{23},z_{32}	
II	53	z_4,z_{24}	
IIIa	53	z_4,z_{24}	
IIIb	53	z_{10}	z
IIIb	53	z_{10}	z_{35}
IIIa	53	z_{29}	
IV	1,53	z_{36},z_{38}	
IIIb	53	z_{52}	z_{35}
IIIb	53	z_{52}	z_{53}
Leda	53		1,6
Group O:54			
Tonev	21,54	b	e,n,x
Winnipeg	54	e,h	1,5
Rossleben	3,54	e,h	1,6
Borreze	54	f,g,s	
Uccle	3,54	g,s,t	
Newholland	4,12,54	m,t	
Poeseldorf	8,20,54	i	z_6
Ochsenwerder	6,7,54	k	1,5
Czernyring	54	r	1,5
Steinwerder	3,15,54	y	1,5
Yerba	54	z_4,z_{23}	
Canton	54	z_{10}	e,n,x
Barry	54	z_{10}	e,n,z_{15}
Group O:55			
II	55	k	z_{39}

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
Group O:56			
II	56	b	
II	56	d	
II	56	e,n,x	1,7
II	56	l,v	z_{39}
II	56	l,z_{28}	
II	56	z	z_6
IIIa	56	z_4,z_{23}	
IIIa	56	z_4,z_{23},z_{32}	
II	56	z_{10}	e,n,x
IIIa	56	z_{29}	
Group O:57			
Antonio	57	a	z_6
II	57	a	z_{42}
Maryland	57	b	1,7
Batonrouge	57	b	e,n,z_{15}
IIIb	57	c	e,n,x,z_{15}
IIIb	57	c	z:[z_{60}]
II	57	d	1,5
II	57	g,[m],s,t	z_{42}
II	57	g,t	
IIIb	57	i	e,n,x,z_{15}
IIIb	57	i	z
IIIb	57	k	e,n,x,z_{15}
IV	57	z_4,z_{23}	
IIIb	57	z_{10}	z
II	57	z_{29}	z_{42}
II	57	z_{39}	e,n,x,z_{15}
II	57	z_{42}	1,6:z_{53}
Group O:58			
II	58	a	z_6
II	58	b	1,5
II	58	c	z_6
II	58	d	z_6
IIIb	58	i	e,n,x,z_{15}
IIIb	58	k	z
IIIb	58	l,v	e,n,x,z_{15}
IIIb	58	l,v	z_{35}
II	58	l,z_{13},z_{28}	1,5
II	58	l,z_{13},z_{28}	z_6
IIIb	58	r	e,n,x,z_{15}
IIIb	58	r	z
IIIb	58	r	z_{53}:[z_{57}]
II	58	z_6	1,6
II	58	z_{10}	1,6
IIIb	58	z_{10}	e,n,x,z_{15}
II	58	z_{10}	z_6
IIIb	58	z_{10}	z_{53}
II	58	z_{39}	e,n,x,z_{15}
IIIb	58	z_{52}	z
IIIb	58	z_{52}	z_{35}
Group O:59			
IIIb	59	c	e,n,x,z_{15}
IIIb	59	i	e,n,x,z_{15}
IIIb	59	i	z
IIIb	59	i	z_{35}
IIIb	59	(k)	e,n,x,z_{15}
II	59	k	(z)
IIIb	59	(k)	z
IIIb	59	(k)	z_{35}
IIIb	59	k	z_{53}
IIIb	59	l,v	z
IIIb	59	l,v	z_{53}
IIIb	59	r	z_{35}
II	1,59	z	z_6
IIIa	59	z_4,z_{23}	
IIIb	59	z_{10}	z_{53}
IIIb	59	z_{10}	z_{57}
IIIa	59	z_{29}	
IIIa	59	z_{36}	
IIIb	59	z_{52}	z_{53}
Group O:60			
II	60	b	
II	60	g,m,t	z_6
IIIb	60	i	e,n,x,z_{15}
IIIb	60	i	z
IIIb	60	i	z_{35}
IIIb	60	k	z
IIIb	60	k	z_{35}
IIIb	60	(k)	z_{53}
IIIb	60	l,v	z
IIIb	60	r	e,n,x,z_{15}
IIIb	60	r	z
IIIb	60	r	z_{35}
IIIb	60	r	z_{53}
II	60	z	e,n,x
IIIb	60	z_{10}	z
IIIb	60	z_{10}	z_{35}
IIIb	60	z_{10}	z_{53}
II	60	z_{29}	e,n,x
V	60	z_{41}	
IIIb	60	z_{52}	1,5,[7]
IIIb	60	z_{52}	z
IIIb	60	z_{52}	z_{35}
IIIb	60	z_{52}	z_{53}
Group O:61			
IIIb	61	c	1,5,(7)
IIIb	61	c	z_{35}
IIIb	61	i	e,n,x,z_{15}
IIIb	61	i	z
IIIb	61	i	z_{35}
IIIb	61	i	z_{53}
IIIb	61	k	1,5,(7)
IIIb	61	k	z_{35}
IIIb	61	(k)	z_{53}
IIIb	61	l,v	1,5,7:[z_{57}]
IIIb	61	l,v	z
IIIb	61	l,v	z_{35}
IIIb	61	r	1,5,7
IIIb	61	r	z
IIIb	61	r	z_{35}
IIIb	61	r	z_{53}
IIIb	61	z_{10}	z_{35}
V	61	z_{35}	
IIIb	61	z_{52}	1,5,7
IIIb	61	z_{52}	z
IIIb	61	z_{52}	z_{35}
IIIb	61	z_{52}	z_{53}
Group O:62			
IIIa	62	g,z_{51}	
IIIa	62	z_4,z_{23}	
IIIa	62	z_4,z_{32}	
IIIa	62	z_{29}	
IIIa	62	z_{36}	
Group O:63			
IIIa	63	g,z_{51}	
IIIa	63	z_4,z_{23}	
IIIa	63	z_4,z_{32}	
IIIa	63	z_{36}	
Group O:65			
IIIb	65	c	1,5,7
IIIb	65	c	z
IIIb	65	c	z_{53}
II	65	g,t	
IIIb	65	i	e,n,x,z_{15}
IIIb	65	(k)	z

(*continued*)

TABLE BXII.γ.262. *(cont.)*

Type[c]	Antigenic formulas[b]		
	Somatic (O) antigen	Flagellar (H) antigen	
		Phase 1	Phase 2
IIIb	65	(k)	z_{35}
IIIb	65	(k)	z_{53}
IIIb	65	l,v	e,n,x,z_{15}
IIIb	65	l,v	z
IIIb	65	l,v	z_{35}
IIIb	65	l,v	z_{53}
IIIb	65	r	z_{35}
IIIb	65	z_{10}	e,n,x,z_{15}
IIIb	65	z_{10}	z
IIIb	65	z_{52}	e,n,x,z_{15}
IIIb	65	z_{52}	z
IIIb	65	z_{52}	z_{35}
IIIb	65	z_{52}	z_{53}
II	65		1,6
Group O:66			
V	66	z_{35}	
V	66	z_{39}	
V	66	z_{41}	
V	66	z_{65}	
V	66	z_{81}	
Group O:67			
Crossness	67	r	1,2

[a]Symbols: [ ], O (not underlined) or H factor that may be present or absent without relation to phage conversion, i.e., factor [5] of O:4 (B) group. When H factors are in square brackets, this means that they are exceptionally found in wild strains. For example, most strains of Paratyphi A possess the monophasic antigen phase 1 H:a. In rare cases, diphasic strains with H:1,5 as H antigen phase 2 may be isolated. For this reason, [1,5] is mentioned in square brackets in the formula of this serovar. ( ), O or H factor weakly agglutinable. The factor (k) corresponding to Arizona factor H:22 is weakly agglutinable by k standard serum, but is normally agglutinable by k polyvalent serum. Symbols for somatic factors determined by phage conversion are underlined (example 6, 14,18). They are present only if the culture is lysogenized by the corresponding converting phage. These factors are usually added to the factors present in nonconverted strain (for example 6,7 →6,7,14). In 0:3,10 group, factors 15 or 15, 34 take the place of factor 10. For this reason, these factors are underlined and quoted into square brackets in this group. These underlined factors are mentioned in the table for serovars in which they were found. It is likely that this situation may be encountered for all serovars in the same group O.

[b]The subfactors of O factors—40, 47, 48, and 50—are no longer mentioned, as their identification is unnecessary in current practice. O and H factors having the same symbol in the Kauffmann–White scheme are always related, but not always identical in different serovars. Table BXII.γ.262 of the antigenic formulae of the *Salmonella* is a scheme established for a diagnostic purpose. Details unnecessary for the diagnosis of serovars are not given in this scheme (e.g., R-phase of the H antigen are not indicated).

[c]Names of the serovars of *S. enterica* subsp. *enterica*. For other subspecies of *S. enterica*, the subspecies to which the serovars belong are indicated by the following symbols: II, serovars of *S. enterica* subsp. *salamae*; IIIa, serovars of *S. enterica* subsp. *arizonae*; IIIb, serovars of *S. enterica* subsp. *diarizonae*; IV, serovars of *S. enterica* subsp. *houtenae*; VI, serovars of *S. enterica* subsp. *indica*; V, serovars of *S. bongori* (the symbol "V" was retained to avoid confusion with serovar name of *S. enterica* subsp. *enterica*).

[d]Strains of this group maybe lysogenized by phage 14(O:6,7, →O:6,7,14). The strains possessing O:6,7,14 had been classified in a special group, C4. They are now classified into group C1. Names formerly given to serovars of group C4 are deleted.

[e]Groups O:6,8(C2) and O:8(C3) differentiated only by presence or absence of O:6 factor, were lumped together in a single group O:8.

[f]Strains of this group maybe lysogenized by phage e15 (O:3,10→ O:3,15), then by phage e34 (O:3,15→ O:3,15,34). In these cases, factors O:15 or O:15,34 take the place of factor O:10 which is no longer agglutinable. The strains of O structure O:3,15 were formerly classified in a special group, E2, and the strains of O structure O:3,15,34 in another group, E3. They are now classified with the strains O:3,10 into group E1. Factors O:15 and O:15,34 are given in square brackets when they have been found in wild strains.

[g]Groups formerly called O:13,22(G1) and O:13,23 (G2) were lumped together in a single group O:13.

Genetics Analysis of 16S rRNA gene sequences indicates that salmonellae belong to the *Gammaproteobacteria* (Chang et al., 1997). *S. enterica* and *S. bongori* (Table BXII.γ.261) were further separated by 16S rDNA analysis and found to be closely related to the *E. coli* and *Shigella* complex by both 16S and 23S rDNA analyses (Christensen et al., 1998).

A total of 1160 genes are located on the genetic map of Typhimurium strain LT2: 1081 are on the circular chromosome, 29 on the 90-kb virulence-associated plasmid, and 50 are not yet mapped (see review by Sanderson et al., 1996). The linkage map of serovar Typhimurium was first determined by F-mediated conjugation. Hfr strains may be selected after F plasmid transfer. Conjugative chromosomal transfer may occur from *Salmonella* to *E. coli*, from *E. coli* to *Salmonella*, and from one serovar of *Salmonella* to another. Chromosomal genes responsible for O, Vi, and H antigens can be transferred from one genus to the other (Iino and Lederberg, 1964). Crosses may be used to localize the regions of the bacterial chromosome that specify avirulence for mice (Krishnapillai and Baron, 1964) or to study the role of O antigen factors in the virulence of *Salmonella* (Mäkelä et al., 1973).

The physical map of the chromosome of several *Salmonella* serovars is now available. Comparisons of these maps show that serovar Typhimurium (Liu and Sanderson, 1992; Liu et al., 1993b), serovar Enteritidis (Liu et al., 1993a), serovar Paratyphi B (Liu et al., 1994a), serovar Paratyphi A (Liu and Sanderson, 1995a), and serovar Typhi (Liu and Sanderson, 1995b) share a common basic genomic structure: genome sizes are all between 4600 and 4800 kb, the order of genes on chromosome segments is usually the same, and all have seven *rrn* operons. Relative to other *Salmonella* serovars, the genomic cleavage map of serovar Paratyphi A shows an insertion of about 100 kb between *rrnH-G* and *proB*, and an inversion of half the genome between *rrnH* and *rrnG*. The chromosome of serovar Typhi has undergone major genetic rearrangements (Liu and Sanderson, 1995b), including (1) homologous recombination between the seven *rrn* operons; (2) inversion that covers the replication terminus region; and (3) at least three insertions, one of which is up to 118 kb long and contains the *viaB* locus for Vi antigen biosynthesis.

As with other *Enterobacteriaceae*, salmonellae may harbor "foreign" replicons (such as temperate phages and plasmids) that may code for virulence determinants, for antibiotic resistance, for antigenic changes of O antigen, and for metabolic characteristics commonly used in diagnostic identification, e.g., lactose or sucrose fermentation (Le Minor et al., 1973, 1974). Thus it is unwise to exclude *Salmonella* solely based on a positive lactose or sucrose reaction. It is also more difficult to identify salmonellae when a pleiotrophic mutation occurs, such as one that simultaneously affects nitrate, tetrathionate, and thiosulfate reductase as well as hydrogenlyase (Le Minor, et al., 1969).

About 5% of *Salmonella* strains produce bacteriocins active against *E. coli*, *Shigella*, and/or *Salmonella* (Fredericq, 1948). Most of these bacteriocins adsorb to the same receptor as that for colicins B, E1, E2, or I. *Salmonella* bacteriocins differ from colicins *sensu stricto* by their activity spectra on colicin indicator strains. Some of these *Salmonella* bacteriocins are not even active against colicin indicator strains but are active against *Salmonella* strains only (Hamon and Péron, 1966).

Susceptibility to the O1 phage Most strains of the genus *Salmonella* are susceptible to the O1 phage of Felix and Callow (1943). This phage is highly specific for *Salmonella*, lysing more than 98% of the strains studied in routine *Salmonella* diagnosis (Kallings, 1967). Susceptibility of *Salmonella* species and subspecies to phage O1 is reported in Table BXII.γ.261. Mutations conferring resistance to O1 phage have been studied by Lindberg and Holme (1969), MacPhee et al. (1975), and Hudson et al. (1978).

A *Salmonella* phage that infects only flagellated bacteria was isolated by Sertic and Boulgakov (1936). Sensitivity to this phage depends on the antigenic specificity of the H antigen. For example, bacteria with antigens of the "G" complex are resistant (Meynell, 1961).

Pathogenicity *Salmonella* serovars may be strictly adapted to one particular host (these serovars are auxotrophic), may be ubiquitous (found in a large number of animal species), or may be of still unknown pathogenicity.

Serovars adapted to humans (e.g., serovar Typhi, serovar Paratyphi A, and serovar Sendai) usually cause severe diseases with septicemia-typhoidic syndrome. They are not naturally pathogenic for other animal species. Salmonellosis is transmitted from person to person, without an intermediate host, through fecal contamination of water and food. The incidence is higher in developing countries with poor hygiene. Typhoid fever, a systemic infection caused by serovar Typhi, remains a major public concern. The World Health Organization has estimated that there are more than 16.6 million typhoid cases per year worldwide, causing 600,000 deaths yearly (Ivanoff and Levine, 1997).

Other serovars are adapted to one animal species: serovar Abortusovis is adapted to sheep and is a major cause of abortion in ewes; serovar Typhisuis and serovar Gallinarum are adapted to swine and poultry, respectively.

Ubiquitous *Salmonella* serovars (e.g., serovar Typhimurium) are mainly responsible for food-borne infections. Low infective doses ($<10^3$) are sufficient to cause clinical symptoms (Blaser and Newman, 1982). Salmonellosis of newborns and infants (who are more susceptible to infections than adults) presents diverse clinical symptoms, from a grave typhoid-like illness with septicemia to a mild or asymptomatic infection. In pediatric wards, the infection is usually transmitted by the hands of personnel.

The entrance of a serovar into a food chain may be the origin of its importation into a country. For example, many countries have become infected with serovar Hadar introduced by imported turkeys, or by serovar Enteritidis introduced by imported poultry and eggs.

After recovery from a clinical case of salmonellosis, some patients, although asymptomatic, remain carriers for weeks, months, or years (i.e., continue to eliminate salmonellae in feces). Carriage contributes to the dissemination of salmonellosis, especially if the diagnosis of the carrier state is not monitored by periodic stool cultures. Antibiotics that are active in curing the disease are usually ineffective in the treatment of the carrier state.

Strains of *Salmonella* from urine are often of the R form. Bilharziosis, a parasitic infection caused by *Schistosoma*, has to be controlled in *Salmonella* carriers (Lo Verde et al., 1980). Sickle-cell anemia must be suspected in cases of osteomyelitis due to *Salmonella* in black children (Vandepitte et al., 1953).

The first step in *Salmonella* pathogenicity is the invasion of the small bowel mucosa. Electron microscopic studies showed that *Salmonella* rapidly adhered to and entered M cells of the follicle-associated epithelium and subsequently invaded absorptive enterocytes. This essential feature of *Salmonella* pathogenesis may be conceptualized as a two-step process. Bacteria may adhere to the target cell first, thereupon inducing an endocytic event that results in internalization of *Salmonella*. The intimate interaction between the bacterium and the host cell appears to trigger cytoskeletal rearrangements and membrane ruffling of the epithelial cell, which ultimately leads to bacterial uptake by macropinocytosis. Complexity of the mechanisms governing *Salmonella* entry into epithelial cells is reflected by the large number of loci involved in this process. Most of them are clustered at centisome 63 of the *Salmonella* chromosome (reviewed by Galan, 1996a, 1996b). In the second step of *Salmonella* pathogenesis, bacteria gain access to the mesenteric lymph nodes, drain through the lymphatics to the thoracic duct into the blood, and ultimately infect the liver and spleen. It is now well established that pathogenic serovars of *Salmonella* other than serovar Typhi contain a plasmid that is essential for systemic dissemination in the appropriate host. Although the role of this virulence-associated plasmid is not fully understood, it is currently thought that plasmid products enhance bacterial growth within the reticuloendothelial system of the host (reviewed by Gulig et al., 1993). In human beings, systemic dissemination of serovar Typhi in the reticuloendothelial system requires expression of the Vi antigen. It was shown that the presence of Vi antigen was associated with resistance to the bactericidal effect of serum, resistance to activation of complement by the alternative pathway, resistance to opsonization by inhibition of C3b binding to bacteria, and resistance to post-phagocytic oxidative burst (reviewed by Virlogeux-Payant and Popoff, 1996).

Antibiotic and drug sensitivity Similarly to *E. coli*, *Salmonella* strains can readily acquire plasmids that contain genes conferring resistance to antibiotics. Multiple resistance is selected for when antibiotics are used extensively in hospitals or added to animal feed. The same plasmids may be found in strains of human or animal origin (Anderson et al., 1975). Since around 1990, strains of serovar Typhi have emerged that are resistant to most previously useful oral antibiotics. The antimicrobials that remain effective are relatively expensive (e.g., fluoroquinolones and ceftriaxone) and some must be administered parentally (e.g., ceftriaxone), thereby posing a quandary for developing countries (reviewed by Ivanoff and Levine, 1997). Similarly, multiple antibiotic-resistant strains of serovar Typhimurium definitive type (DT) 104 have emerged recently (Wall et al., 1994; Glynn et al., 1998; Poppe et al., 1998). They are pathogenic for humans and animals, particularly cattle. The DT104 strains were isolated in the United States, Canada, and Europe. More prudent use of antimicrobial agents in farm animals and more effective disease prevention on farms are necessary to reduce the dissemination of this multidrug-resistant pathogen (Glynn et al., 1998).

Ecology Although some *Salmonella* serovars are strictly host adapted, the majority have a wide host range (e.g., serovar Typhimurium). Some are localized in a particular region of the globe (e.g., serovar Sendai in the Far East, serovar Berta in North America), but others are ubiquitous (e.g., serovar Typhimurium). Strains belonging to *S. enterica* subsp. *salamae*, subsp. *arizonae*, and subsp. *diarizonae* are frequently isolated from the intestinal contents of cold-blooded animals and only rarely from human beings and warm-blooded animals. Strains of subsp. *houtenae* and *S. bongori* are isolated chiefly from the environment and are rarely pathogenic for humans.

Enrichment and Isolation Procedures

Isolation from blood is done according to the classical method for hemoculture. A biphasic culture bottle containing a vertical agar layer along one side and a broth medium at the bottom (Castaneda, 1947; Hall et al., 1979; Krieg and Gerhardt, 1981) prepared with tryptic soy agar/broth containing 2% sodium citrate is convenient. Isolated colonies grow on the agar layer. Identification is usually done by (a) diagnosis of the family *Enterobacteriaceae*, (b) diagnosis of the genus *Salmonella* (diagnosis of the subspecies for strains isolated from blood cultures is not

routinely necessary, because almost all blood isolates belong to subsp. *enterica*), (c) diagnosis of the serovar, (d) determination of the antibiotic susceptibility pattern, and (e) further study of the biovar and phagovar if indicated.

Selective procedures are needed for the isolation of *Salmonella* from specimens containing mixed bacterial flora (fecal samples, autopsy samples, food, environmental samples, etc.). Enrichment (i.e., an increased ratio of *Salmonella* cells to other bacterial cells during incubation) is obtained using liquid nutrient media containing selective agents that inhibit or retard growth of bacteria other than *Salmonella*. Use of enrichment media is essential when the number of salmonellae in a sample is very low, i.e., when the probability of finding colonies by direct isolation is low. Three media may be recommended for general use: (a) the tetrathionate medium of Muller (1923); (b) Muller's medium modified by Kauffmann (1935) by addition of bile and brilliant green; and (c) selenite F broth devised by Leifson (1936). Tetrathionate and selenite broth are suitable for all *Salmonella* serovars. Tetrathionate bile brilliant-green medium is suitable for all serovars, except host-adapted serovars such as serovar Typhi. Enrichment media should be heavily inoculated, e.g., 0.5 ml of fecal suspension per 10 ml of medium. After incubation for 18 h at 37°C, a loopful of enrichment culture is streaked onto agar plating medium.

The same enrichment media may be used for detection of salmonellae in water. The simplest method is to add one volume of the water sample to an equal volume of double-strength medium. For detecting salmonellae in food, a generally suitable procedure is to inoculate 25 g of the suspected food into 225 ml of selenite F broth, incubate for 24 h, and isolate on selective agar media. In the case of a dehydrated food, nutrient broth containing the sample is incubated overnight before inoculation of enrichment media.

Agar media are used for isolation of salmonellae. Streaking a loopful of enrichment culture or a suspension of the sample (e.g., stool) should be done carefully to obtain the greatest number of perfectly isolated colonies. Because the most discriminating character is lactose fermentation, the majority of media for isolation contain lactose and a pH indicator. In addition, the media contain selective agents to inhibit the growth of non-*Salmonella* organisms and the swarming of *Proteus mirabilis* and *P. vulgaris*. Some media also contain ferrous citrate detection of H_2S-producing bacteria.

Examples of media of moderate selectivity are (a) MacConkey agar, which contains lactose, neutral red, and the selective inhibitors crystal violet and bile salts; lactose-positive colonies are red, lactose-negative colonies are colorless; and (b) desoxycholate citrate agar, which contains lactose, neutral red, and the selective agent desoxycholate; ferric ammonium citrate is included as an indicator of H_2S production; lactose-positive colonies are red; lactose-negative colonies are colorless; if H_2S is produced, the inner part of the colony is black.

Examples of media of higher selectivity are the following: (a) Salmonella–Shigella (SS) agar, which contains lactose, neutral red, and the selective agents brilliant green and bile salts. Ferric citrate is an indicator of H_2S production. The appearance of colonies is the same as on desoxycholate citrate agar. (b) Brilliant green agar, which contains lactose, phenol red, and the selective agent brilliant green. This medium is easy to prepare and is suitable for all salmonellae except host-adapted serovars. It is not suitable for shigellae. Lactose-positive colonies are green, lactose-negative colonies are pink. All of the above-mentioned media were reviewed by Kauffmann (1966) and Edwards and Ewing (1972). (c) Hektoen enteric medium (King and Metzger, 1968), which contains lactose, sucrose and salicin, a mixture of bromothymol blue and Andrade's pH indicator, ferric citrate to detect H_2S production, and sodium desoxycholate as a selective inhibitor. Colonies that do not ferment any of the three sugars (e.g., *Salmonella*) are blue-green, with a black center if H_2S is produced. Colonies fermenting one or more of the sugars (e.g., *E. coli*, *Enterobacter cloacae*) are salmon-colored. This medium is suitable for all *Salmonella* serovars and for shigellae.

A general procedure for the detection of salmonellae in feces or food is as follows. A suspension of the sample in saline is streaked onto the chosen isolation medium and also inoculated into an enrichment broth. After overnight incubation, the plating medium is examined for suspect colonies (lactose negative, H_2S positive or negative); also, a loopful of the enrichment culture is streaked onto another plate of selective agar medium. After overnight incubation, this plate is also examined for suspect colonies. A quick screening of several suspect colonies is done by inoculating each into a few drops of urea medium and incubating at 37°C for 2 h. Biochemical characterization is continued only for urease-negative colonies (urease-positive colonies growing at 18 h are likely to be *Proteus*) by inoculating, e.g., triple sugar–iron (TSI) or Kligler-Hajna medium. *Salmonella* must be differentiated mainly from *Citrobacter freundii*, *P. mirabilis*, and, in food bacteriology, *Alteromonas putrefaciens*. To detect *Salmonella* belonging to subsp. *arizonae* and serovar Diarizonae, attention should be given to lactose-positive, H_2S-positive colonies on plating media.

Maintenance Procedures

Salmonella cultures remain viable for many years when stored on peptone agar (meat extract, 5.0 g; peptone, 10.0 g; NaCl, 3.0 g; $Na_2HPO_4 \cdot 12H_2O$, 2.0 g; agar, 10.0 g; distilled water, 1000 ml; pH 7.4) and distributed into small, tightly stoppered, screw-capped tubes. This medium is stab-inoculated and kept in the dark at room temperature. Lyophilization and freezing at or below −70°C give very good results. For lyophilization, it is necessary to isolate each subculture and to select a colony with the desired serologic characteristics.

Differentiation of the genus *Salmonella* from other genera

Characteristics useful for differentiating the genus *Salmonella* from other *Enterobacteriaceae* are given in Tables BXII.γ.193, BXII.γ.194, and BXII.γ.196 of the chapter on the family *Enterobacteriaceae*.

Taxonomic Comments

The species concept in the genus *Salmonella* has evolved in several overlapping phases. The typhoid bacillus was first observed by Eberth (1880) in spleen sections and mesenteric lymph nodes from a patient who died from typhoid fever. During the following decade, other bacteria were isolated from clinical cases of typhoid fever, but they were distinct from the typhoid bacillus both culturally and serologically. The genus *Salmonella* was created by Lignières (1900). Hence, *Salmonella* strains were considered different species when isolated from different conditions or different hosts. However, it was soon realized that a number of these so-called species were ubiquitous, and emphasis shifted to antigenic properties.

Serological analysis of *Salmonella* O and H antigens was initiated by White (1926). This work was extended by Kauffmann (1961), who defined species as "a group of related sero-fermentative phage types". Consequently, each *Salmonella* serovar was considered as a species. But, as most serovars could not be distinguished by biochemical tests, Kauffmann (1966) divided the

genus *Salmonella* into four subgenera (I–IV) and continued to apply his concept of "one serovar–one species". Nevertheless, phenotypic studies and numerical taxonomy showed that, apart from host-adapted serovars, *Salmonella* serovars within each subgenus were biochemically indistinguishable (reviewed by Le Minor and Popoff, 1987). For this reason, Kauffmann's subgenera were considered to be species (Le Minor et al., 1970b).

Later, DNA relatedness studies demonstrated that all *Salmonella* serovars formed a single DNA hybridization group with seven subgroups delineated by studies of the thermal stability of hybrids (Crosa et al., 1973a; Stoleru et al., 1976; Le Minor et al., 1986). The seven DNA subgroups could be differentiated by using biochemical characteristics. Correlation between DNA subgroups and Kauffmann's subgenera was close except that subgenus III was split into two DNA subgroups containing monophasic and diphasic serovars, respectively.

Based on these numerical taxonomy and DNA relatedness studies, it was proposed that the genus *Salmonella* should consist of a single species that could be divided into seven subspecies (I–VII), although subspecies V might possibly represent a second species (Le Minor et al., 1982, 1986). The strict application of the Bacteriological Code (Rules Revision Committee, Judicial Commission, International Committee on Systematic Bacteriology, 1985) led Le Minor et al. (1982, 1986) to propose *S. choleraesuis* as the species name, having seven subspecies designated subsp. *choleraesuis*, subsp. *salamae*, subsp. *arizonae*, subsp. *diarizonae*, subsp. *houtenae*, subsp. *bongori*, and subsp. *indica*. Subspecies names were validated by announcement in the *International Journal of Systematic Bacteriology* (1985, 1987). However, the name *S. choleraesuis* could be considered as an ambiguous name since this name was used for the serovar possessing the antigenic formula 6,7:c:1,5 (Table BXII.γ.262). To avoid further confusion, it was proposed that the type species of the genus *Salmonella* be *S. enterica* (Le Minor and Popoff, 1987). This name, first proposed by Kauffmann and Edwards (1952), received unanimous support from the members of the Subcommittee of *Enterobacteriaceae* of the International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology (Manchester, U.K., 1986). However, the request to change the name of the type species to *S. enterica* was not approved by the Judicial Commission. A second request has been made and, in the interim, the WHO collaborating center for reference and research on *Salmonella* and national *Salmonella* reference laboratories are using *S. enterica*.

Finally, by using multilocus enzyme analysis, it was shown that *S. enterica* subsp. *bongori* had evolved considerably from other subspecies (Reeves et al., 1989a). Based on this divergence and on DNA relatedness data (Le Minor et al., 1982; 1986), Reeves et al. (1989a) proposed that *S. enterica* subsp. *bongori* be elevated to the level of species in the new combination *S. bongori*.

The practice of giving names to the serovars of *S. enterica* subsp. *enterica* should continue since the diagnostic use of the Kauffmann–White scheme (Table BXII.γ.262) is overridingly important and since these names are very familiar to microbiologists and physicians. However, these names must no longer be italicized and the first letter must be a capital. Serovars of the other subspecies of *S. enterica* and those of *S. bongori* should be designated only by their antigenic formulae.

Differentiation of the species of the genus *Salmonella*

Table BXII.γ.261 presents the biochemical characteristics differentiating the species and subspecies of the genus *Salmonella*. The antigenic formulae of *Salmonella* serovars (i.e., the Kauffmann–White scheme) is given in Table BXII.γ.262. An alphabetical listing of serovars indicating the O groups is presented in Table BXII.γ.263.

List of species of the genus Salmonella

1. **Salmonella enterica** (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987, 466.
en.te.ri' ca. Gr. n. *enteron* gut; L. adj. *enterica* of the gut.

The description of *S. enterica* is that of *S. choleraesuis sensu* (Le Minor et al., 1982). The characteristics are as described for the genus (see Tables BXII.γ.193, BXII.γ.194, and BXII.γ.196 of the chapter on the family *Enterobacteriaceae* and as listed in Table BXII.γ.261. *S. enterica* can be divided into six subspecies (Le Minor et al., 1982, 1986; Reeves et al. 1989a).

The type strain carries a virulence-associated plasmid of about 90 kb. Antigenic formula 4,5,12:i:1,2 (serovar Typhimurium).

The mol% G + C of the DNA is: 50–53 (hydrolysis and chromatography, Bd, T_m).

Type strain: LT2, ATCC 43971, CIP 60.62, NCIB 11450.

a. **Salmonella enterica** *subsp.* **enterica** (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987, 467.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 1443 serovars (Popoff et al., 1998). Isolated mainly from humans and warm-blooded animals.

A list of selected serovars belonging to *S. enterica* subsp. *enterica* is given below.

The mol% G + C of the DNA is: see species description.

Type strain: LT2, ATCC 43971, CIP 60.62, NCIB 11450.

i. **Salmonella enterica** *subsp.* **enterica** *serovar* **Choleraesuis** (Smith 1894) Weldin 1927, 155 (*Bacillus cholerae suis* Smith 1894, 9.)
chol.er.ae.su' is. Gr. n. *cholera* cholera; L. n. *sus* hog. M.L. gen. n. *suis* of a hog; M.L. gen. n. *choleraesuis* of hog cholera.

Antigenic formula: 6,7:c:1,5. The detailed O antigen formula is normally 6_2,7, but this may be transformed by lysogenization into 6_1,7 or 6_2,7,14. Arabinose and trehalose are not fermented; dulcitol is slowly and irregularly fermented. Those strains that produce H_2S are designated as serovar Choleraesuis biovar Kunzendorf. Pathogenic for humans and animals.

The mol% G + C of the DNA is: see species description.

Deposited strain: ATCC 13312, NCTC 5735.

ii. **Salmonella enterica** *subsp.* **enterica** *serovar* **Enteritidis** (Gaertner 1888) Castellani and Chalmers 1919, 939 (*Bacterium enteritidis* Gaertner 1888, 573.)
en.te.ri'ti.dis. Gr. n. *enteron* gut, intestine; M.L. n. *enteritis* enteritis, inflammation of the intestine; M.L. gen. n. *enteritidis* of enteritis.

Antigenic formula: 1,9,12:g,m:−. The presence of factor O1 is connected with lysogenization. Ubiquitous and frequently the cause of infections in humans and

TABLE BXII.γ.263. Alphabetical list of names of *Salmonella* serovars, indicating the O groups

Serovar	O Group
Aachen	J
Aarhus	K
Aba	C2–C3
Abadina	M
Abaetetuba	F
Aberdeen	F
Abidjan	Q
Ablogame	I
Abobo	I
Abony	B
Abortusequi	B
Abortusovis	B
Abuja	F
Accra	E4
Ackwepe	D2
Adabraka	E1
Adamstown	M
Adamstua	F
Adana	U
Adelaide	O
Adeoyo	I
Aderike	M
Adime	C1
Adjame	G
Aequatoria	C1
Aesch	C2–C3
Aflao	H
Africana	B
Afula	C1
Agama	B
Agbara	I
Agbeni	G
Agege	E1
Ago	N
Agodi	O
Agona	B
Agoueve	G
Ahanou	J
Ahepe	U
Ahmadi	E4
Ahoutoue	S
Ahuza	U
Ajiobo	G
Akanji	C2–C3
Akuafo	I
Alabama	D1
Alachua	O
Alagbon	C2–C3
Alamo	C1
Albany	C2–C3
Albert	B
Albuquerque	H
Alexanderplatz	X
Alexanderpolder	C2–C3
Alfort	E1
Alger	P
Alkmaar	E4
Allandale	R
Allerton	E1
Alma	Q
Alminko	C2–C3
Altendorf	B
Altona	C2–C3
Amager	E1
Amba	F
Amersfoort	C1
Amherstiana	C2–C3
Amina	I
Aminatu	E1
Amounderness	E1
Amoutive	M
Amsterdam	E1
Amunigun	I
Anatum	E1
Anderlecht	E1
Anecho	O
Anfo	Q
Angers	C2–C3
Angoda	N
Angouleme	I
Ank	M
Anna	G
Annedal	I
Antarctica	D1
Antonio	57
Antsalova	51
Antwerpen	T
Apapa	W
Apeyeme	C2–C3
Aprad	W
Aqua	N
Aragua	N
Arapahoe	H
Arechavaleta	B
Arusha	U
Aschersleben	N
Ashanti	M
Assen	L
Assinie	E1
Asylanta	E1
Atakpame	C2–C3
Atento	F
Athinai	C1
Ati	F
Augustenborg	C1
Austin	C1
Australia	Y
Avignon	I
Avonmouth	E4
Ayinde	B
Ayton	B
Azteca	B
Babelsberg	M
Badagry	I
Baguida	L
Baguirmi	N
Bahati	G
Bahrenfeld	H
Baiboukoum	C1
Baildon	D2
Bakau	M
Balcones	W
Balili	M
Ball	B
Bama	J
Bamboye	D2
Bambylor	D2
Banalia	C2–C3
Banana	B
Banco	M
Bandia	O
Bangkok	P
Bangui	D1
Banjul	H
Bardo	C2–C3
Bareilly	C1
Bargny	C2–C3
Barmbek	I
Barranquilla	I
Barry	54
Basingstoke	D2

(*continued*)

TABLE BXII.γ.263. *(cont.)*

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Bassa	C2–C3
Bassadji	M
Batonrouge	57
Battle	I
Bazenheid	C2–C3
Be	C2–C3
Beaudesert	H
Bedford	E4
Belem	C2–C3
Belfast	C2–C3
Bellevue	C2–C3
Benfica	E1
Benguella	R
Benin	D2
Benue	C2–C3
Bere	X
Bergedorf	D2
Bergen	X
Bergues	51
Berkeley	U
Berlin	J
Berta	D1
Bessi	E1
Bethune	E4
Biafra	E1
Bida	E4
Bietri	N
Bignona	J
Bijlmer	R
Bilu	E4
Binche	X
Bingerville	X
Binningen	W
Birkenhead	C1
Birmingham	E1
Bispebjerg	B
Bissau	B
Blegdam	D1
Blijdorp	H
Blitta	X
Blockley	C2–C3
Bloomsbury	E1
Blukwa	K
Bobo	V
Bochum	B
Bodjonegoro	N
Boecker	H
Bofflens	S
Bokanjac	M
Bolama	V
Bolombo	E1
Bolton	E1
Bonames	J
Bonariensis	C2–C3
Bonn	C1
Bootle	X
Borbeck	G
Bordeaux	52
Borreze	54
Borromea	T
Bouake	I
Bournemouth	D1
Bousso	H
Bovismorbificans	C2–C3
Bracknell	G
Bradford	B
Braenderup	C1
Brancaster	B
Brandenburg	B
Brazil	I
Brazos	K
Brazzaville	C1
Breda	C2–C3
Bredeney	B
Brefet	V
Breukelen	C2–C3
Brevik	I
Brezany	B
Brijbhumi	F
Brikama	C2–C3
Brindisi	F
Brisbane	M
Bristol	G
Brive	T
Broc	T
Bron	G
Bronx	C2–C3
Brooklyn	I
Broughton	E4
Bruck	C1
Brunei	C2–C3
Brunflo	I
Bsilla	C2–C3
Buckeye	Y
Budapest	B
Bukavu	R
Bukuru	C2–C3
Bulgaria	C2–C3
Bullbay	F
Bulovka	C1
Burgas	I
Burundi	S
Bury	B
Businga	C1
Butantan	E1
Butare	52
Buzu	H
Caen	I
Cairina	E1
Cairns	W
Calabar	E4
California	B
Camberene	O
Camberwell	D1
Campinense	D1
Canada	B
Canary	R
Cannobio	M
Cannonhill	E4
Cannstatt	E4
Canton	54
Caracas	H
Cardoner	I
Carmel	J
Carnac	K
Carno	E4
Carrau	H
Carswell	V
Casablanca	W
Casamance	R
Catalunia	M
Catanzaro	H
Cayar	C1
Cerro	K
Ceyco	D2
Chagoua	G
Chailey	C2–C3
Champaign	Q
Chandans	F
Charity	H
Charlottenburg	C2–C3
Cheltenham	D2

(*continued*)

TABLE BXII.γ.263. *(cont.)*

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Chester	B
Chicago	M
Chichester	E4
Chichiri	H
Chile	C1
Chincol	C2–C3
Chingola	F
Chiredzi	F
Chittagong	E4
Choleraesuis	C1
Chomedey	C2–C3
Christiansborg	V
Clackamas	B
Claibornei	D1
Clanvillian	F
Clerkenwell	E1
Cleveland	C2–C3
Cochin	D2
Cocody	C2–C3
Coeln	B
Coleypark	C1
Colindale	C1
Colobane	F
Colombo	P
Colorado	C1
Concord	C1
Congo	G
Connecticut	F
Coogee	T
Coquilhatville	E1
Coromandel	C1
Corvallis	C2–C3
Cotham	M
Cotia	K
Cremieu	C2–C3
Crewe	F
Croft	M
Crossness	67
Cubana	G
Cuckmere	E1
Cullingworth	M
Cumberland	Q
Curacao	C2–C3
Cyprus	C2–C3
Czernyring	54
Daarle	C2–C3
Dabou	C2–C3
Dadzie	51
Dahlem	Y
Dahomey	X
Dahra	J
Dakar	M
Dakota	I
Dallgow	E4
Damman	C1
Dan	51
Dapango	X
Daula	C2–C3
Daytona	C1
Deckstein	D2
Delmenhorst	K
Dembe	O
Demerara	G
Denver	C1
Derby	B
Derkle	52
Dessau	E4
Deversoir	W
Dibra	M
Dieuppeul	M
Diguel	G

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Diogoye	C2–C3
Diourbel	L
Djakarta	Y
Djama	T
Djelfa	C2–C3
Djermaia	M
Djibouti	J
Djugu	C1
Doba	D2
Doel	M
Doncaster	C2–C3
Donna	N
Doorn	M
Douala	M
Dougi	Z
Doulassame	N
Dresden	M
Driffield	R
Drogana	B
Dublin	D1
Duesseldorf	C2–C3
Dugbe	W
Duisburg	B
Dumfries	E1
Dunkwa	C2–C3
Durban	D1
Durham	G
Duval	R
Ealing	O
Eastbourne	D1
Eastglam	E4
Eberswalde	M
Eboko	C2–C3
Ebrie	O
Echa	P
Edinburg	C1
Edmonton	C2–C3
Egusi	S
Egusitoo	T
Eingedi	C1
Eko	B
Ekotedo	D2
Ekpoui	X
Elisabethville	E1
Elokate	D1
Elomrane	D1
Emek	C2–C3
Emmastad	P
Encino	H
Enschede	O
Entebbe	B
Enteritidis	D1
Enugu	I
Epicrates	E1
Epinay	F
Eppendorf	B
Escanaba	C1
Eschberg	D1
Eschweiler	C1
Essen	B
Etterbeek	F
Euston	F
Everleigh	E1
Ezra	M
Fairfield	M
Fajara	M
Faji	T
Falkensee	E1
Fallowfield	E1
Fann	F
Fanti	G

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Farakan	M
Farcha	U
Fareham	E4
Farmsen	G
Farsta	B
Fass	Z
Fayed	C2–C3
Ferlo	S
Ferruch	C2–C3
Finaghy	B
Findorff	F
Finkenwerder	H
Fischerhuette	I
Fischerkietz	H
Fischerstrasse	V
Fitzroy	Y
Florian	E1
Florida	H
Flottbek	52
Fluntern	K
Fomeco	W
Fortlamy	I
Fortune	B
Franken	D1
Frankfurt	I
Freefalls	M
Freetown	P
Freiburg	E1
Fresno	D2
Friedenau	G
Friedrichsfelde	M
Frintrop	D1
Fufu	E1
Fulda	E4
Fulica	B
Fyris	B
Gabon	C1
Gafsa	I
Galiema	C1
Galil	E1
Gallen	F
Gallinarum	D1
Gamaba	V
Gambaga	L
Gambia	O
Gaminara	I
Garba	H
Garoli	C1
Gassi	O
Gateshead	D2
Gatineau	E4
Gatow	C1
Gatuni	C2–C3
Gbadago	E1
Gdansk	C1
Gege	N
Georgia	C1
Gera	T
Geraldton	D2
Gerland	I
Ghana	L
Giessen	N
Give	E1
Giza	C2–C3
Glasgow	I
Glidji	F
Glostrup	C2–C3
Gloucester	B
Gnesta	E4
Godesberg	N
Goelzau	E1

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Goeteborg	D1
Goettingen	D1
Gokul	51
Goldcoast	C2–C3
Goma	C1
Gombe	C1
Good	L
Gori	J
Goulfey	R
Gouloumbo	O
Goverdhan	D1
Gozo	M
Grampian	C1
Grancanaria	I
Grandhaven	N
Granlo	J
Graz	U
Greiz	R
Groenekan	K
Grumpensis	G
Guarapiranga	N
Guerin	D2
Guildford	M
Guinea	V
Gustavia	F
Gwale	T
Gwoza	E4
Haardt	C2–C3
Hadar	C2–C3
Hadejia	J
Haduna	B
Haelsingborg	C1
Haferbreite	T
Haga	O
Haifa	B
Halle	M
Hallfold	B
Handen	G
Hann	R
Hannover	I
Haouaria	G
Harburg	H
Harcourt	51
Harleystreet	E1
Harrisonburg	E1
Hartford	C1
Harvestehude	T
Hatfield	M
Hato	B
Havana	G
Hayindogo	E4
Heerlen	F
Hegau	Q
Heidelberg	B
Hemingford	Z
Hennekamp	T
Hermannswerder	M
Heron	I
Herston	C2–C3
Herzliya	F
Hessarek	B
Hidalgo	C2–C3
Hiduddify	C2–C3
Hillegersberg	D2
Hillingdon	D2
Hillsborough	C1
Hilversum	N
Hindmarsh	C2–C3
Hisingen	Y
Hissar	C1
Hithergreen	I

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Hoboken	E1
Hofit	Q
Hoghton	E1
Holcomb	C2–C3
Homosassa	H
Honelis	M
Hongkong	E4
Horsham	H
Huddinge	E1
Huettwillen	B
Hull	I
Huvudsta	E1
Hvittingfoss	I
Hydra	L
Ibadan	G
Ibaragi	L
Idikan	G
Ikayi	E1
Ikeja	M
Ilala	M
Ilugun	E4
Imo	W
Inchpark	C2–C3
India	D2
Indiana	B
Infantis	C1
Inganda	C1
Inglis	D2
Inpraw	S
Inverness	P
Ipeko	D1
Ipswich	S
Irchel	D2
Irenea	J
Irigny	U
Irumu	C1
Isangi	C1
Isaszeg	Y
Israel	D1
Istanbul	C2–C3
Istoria	H
Isuge	G
Itami	D1
Ituri	B
Itutaba	D2
Ivory	I
Ivorycoast	Z
Jaffna	D1
Jalisco	F
Jamaica	D1
Jambur	L
Jangwani	J
Javiana	D1
Jedburgh	E1
Jericho	B
Jerusalem	C1
Joal	E1
Jodhpur	W
Johannesburg	R
Jos	B
Juba	E4
Jubilee	J
Jukestown	G
Kaapstad	B
Kabete	51
Kaduna	C1
Kahla	T
Kainji	E4
Kaitaan	H
Kalamu	B
Kalina	E1

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Kallo	C2–C3
Kalumburu	C2–C3
Kambole	C1
Kamoru	B
Kampala	T
Kande	E4
Kandla	J
Kaneshie	T
Kanifing	H
Kano	B
Kaolack	X
Kapemba	D1
Karachi	W
Karamoja	R
Karaya	51
Karlshamn	J
Kasenyi	P
Kassberg	H
Kedougou	G
Kentucky	C2–C3
Kenya	C1
Kermel	V
Keve	L
Kiambu	B
Kibi	I
Kibusi	M
Kidderminster	P
Kiel	A
Kikoma	I
Kimberley	P
Kimpese	D1
Kimuenza	B
Kindia	E4
Kingabwa	U
Kingston	B
Kinondoni	J
Kinson	E4
Kintambo	G
Kirkee	J
Kisangani	B
Kisarawe	F
Kisii	C1
Kitenge	M
Kivu	C1
Klouto	P
Kodjovi	X
Koenigstuhl	B
Koessen	A
Kofandoka	W
Koketime	V
Kokoli	N
Kokomlemle	Q
Kolar	D2
Kolda	C2–C3
Konolfingen	M
Konstanz	C2–C3
Korbol	C2–C3
Korlebu	E4
Korovi	P
Kortrijk	C1
Kottbus	C2–C3
Kotte	C1
Kotu	D1
Kouka	E4
Koumra	C1
Kpeme	M
Kraligen	C2–C3
Krefeld	E4
Kristianstad	E1
Kua	V
Kubacha	B

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Kuessel	M
Kumasi	N
Kunduchi	B
Kuntair	H
Kuru	C2–C3
Labadi	C2–C3
Lagos	B
Lamberhurst	E1
Lamin	E1
Lancaster	J
Landala	S
Landau	N
Landwasser	E1
Langenhorn	K
Langensalza	E1
Langford	M
Lansing	P
Laredo	H
Larochelle	C1
Lattenkamp	W
Lawndale	D1
Lawra	V
Leatherhead	S
Lechler	51
Leda	53
Leer	K
Leeuwarden	F
Legon	B
Leiden	G
Leipzig	S
Leith	C2–C3
Lekke	E1
Lene	F
Leoben	M
Leopoldville	C1
Lerum	E4
Lexington	E1
Lezennes	C2–C3
Libreville	M
Ligeo	N
Ligna	O
Lika	C1
Lille	C1
Limete	B
Lindenburg	C2–C3
Lindern	H
Lindi	P
Linguere	D2
Lingwala	I
Linton	G
Lisboa	I
Lishabi	D2
Litchfield	C2–C3
Liverpool	E4
Livingstone	C1
Livulu	N
Ljubljana	B
Llandoff	E4
Llobregat	V
Loanda	C2–C3
Lockleaze	C1
Lode	J
Lodz	S
Loenga	T
Logone	Q
Lokstedt	E4
Lomalinda	D1
Lome	D1
Lomita	C1
Lomnava	I
London	E1
Losangeles	I
Loubomo	B
Louga	N
Louisiana	D2
Lovelace	G
Lowestoft	J
Lubumbashi	S
Luciana	F
Luckenwalde	M
Luedinghausen	J
Luke	X
Lutetia	51
Lyon	X
Maastricht	F
Macallen	E1
Macclesfield	D2
Machaga	E4
Madelia	H
Madiago	E4
Madigan	V
Madison	L
Madjorio	E1
Madras	B
Magherafelt	C2–C3
Magumeri	H
Magwa	L
Mahina	D2
Maiduguri	E4
Makiling	U
Makiso	C1
Malakal	I
Malaysia	M
Malika	V
Malmoe	C2–C3
Malstatt	I
Mampeza	H
Mampong	G
Manchester	C2–C3
Mandera	I
Mango	P
Manhattan	C2–C3
Mannheim	F
Mapo	C2–C3
Mara	Q
Maracaibo	F
Marburg	G
Maricopa	T
Marienthal	E1
Maritzburg	V
Maron	E1
Maroua	F
Marseille	F
Marshall	G
Maryland	57
Marylebone	D2
Masembe	E1
Maska	B
Massakory	O
Massenya	B
Matadi	J
Mathura	D2
Matopeni	N
Mayday	D2
Mbandaka	C1
Mbao	U
Meekatharra	W
Melbourne	T
Meleagridis	E1
Memphis	K
Menden	C1
Mendoza	D1

(*continued*)

TABLE BXII.γ.263. *(cont.)*

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Menston	C1
Mesbit	X
Meskin	51
Messina	N
Mgulani	P
Miami	D1
Michigan	J
Middlesbrough	T
Midway	H
Mikawasima	C1
Millesi	R
Milwaukee	U
Mim	G
Minna	H
Minnesota	L
Mishmarhaemek	G
Mississippi	G
Missouri	F
Miyazaki	D1
Mjordan	N
Mkamba	C1
Mocamedes	M
Moero	M
Moers	F
Mokola	E1
Molade	C2–C3
Molesey	52
Mono	B
Mons	B
Monschaui	O
Montevideo	C1
Montreal	U
Morehead	N
Morillons	M
Morningside	N
Mornington	H
Morocco	N
Morotai	J
Moroto	M
Moscow	D1
Moualine	X
Moundou	51
Mountmagnet	L
Mountpleasant	X
Moussoro	H
Mowanjum	C2–C3
Mpouto	I
Muenchen	C2–C3
Muenster	E1
Muguga	V
Mulhouse	D1
Mundonobo	M
Mura	B
Naestved	D1
Nagoya	C2–C3
Nakuru	B
Namibia	C1
Namoda	X
Nanergou	C2–C3
Nanga	G
Nantes	D2
Napoli	D1
Narashino	C2–C3
Nashua	M
Natal	D1
Naware	I
Nchanga	E1
Ndjamena	H
Ndolo	D1
Neftenbach	B
Nessa	H

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Nessziona	C1
Neudorf	N
Neukoelln	C1
Neumuenster	B
Neunkirchen	P
Newholland	54
Newlands	E1
Newmexico	D1
Newport	C2–C3
Newrochelle	E1
Newyork	G
Ngaparou	D2
Ngili	C1
Ngor	E4
Niakhar	V
Niamey	J
Niarembe	V
Niederoderwitz	U
Nieukerk	C1
Nigeria	C1
Nijmegen	N
Nikolaifleet	I
Niloese	E4
Nima	M
Nimes	G
Nitra	A
Niumi	E4
Njala	P
Nola	C1
Nordrhein	D2
Nordufer	C2–C3
Norton	C1
Norwich	C1
Nottingham	I
Nowawes	R
Noya	C2–C3
Nuatja	I
Nyanza	F
Nyborg	E1
Nyeko	I
Oakey	C1
Oakland	C1
Obogu	C1
Ochiogu	E4
Ochsenwerder	54
Ockenheim	N
Odienne	R
Odozi	N
Oerlikon	Q
Oersterbro	E4
Offa	S
Ogbete	U
Ohio	C1
Ohlstedt	E1
Okatie	G
Okefoko	E1
Okerara	E1
Oldenburg	I
Olten	D2
Omifisan	R
Omuna	C1
Ona	M
Onarimon	D1
Onderstepoort	H
Onireke	E1
Ontario	D2
Oran	P
Oranienburg	C1
Orbe	T
Ord	52
Ordonez	G

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Orientalis	I
Orion	E1
Oritamerin	C1
Orlando	K
Orleans	U
Os	D1
Oskarshamn	M
Oslo	C1
Osnabrueck	F
Othmarschen	C1
Ottawa	D1
Ouakam	D2
Oudwijk	G
Overchurch	R
Overschie	51
Overvecht	N
Oxford	E1
Oyonnax	C1
Pakistan	C2–C3
Palamaner	V
Palime	C1
Panama	D1
Papuana	C1
Paratyphi A	A
Paratyphi B	B
Paratyphi C	C1
Paris	C2–C3
Parkroyal	E4
Pasing	B
Patience	M
Penarth	D1
Penilla	M
Pensacola	D1
Perth	P
Petahtikve	E4
Phaliron	C2–C3
Pharr	F
Pietersburg	E1
Pisa	I
Planckendael	C1
Ploufragan	V
Plymouth	D2
Poano	H
Poeseldorf	54
Poitiers	C1
Pomona	M
Pontypridd	K
Poona	G
Portanigra	C2–C3
Portland	D1
Potengi	K
Potosi	H
Potsdam	C1
Potto	D2
Powell	D1
Praha	C2–C3
Pramiso	E1
Presov	C2–C3
Preston	B
Pretoria	F
Putten	G
Quebec	V
Quentin	D2
Quinhon	X
Quiniela	C2–C3
Ramatgan	N
Ramsey	M
Raus	G
Rawash	K
Reading	B
Rechovot	C2–C3

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Redba	C1
Redhill	F
Redlands	I
Regent	E1
Reinickendorf	B
Remete	F
Remiremont	C2–C3
Remo	B
Reubeuss	C2–C3
Rhone	L
Rhydyfelin	I
Richmond	C1
Rideau	E4
Ridge	D1
Ried	G
Riggil	C1
Riogrande	R
Rissen	C1
Rittersbach	P
Riverside	W
Roan	P
Rochdale	Z
Rogy	M
Romanby	G
Roodepoort	G
Rossleben	54
Rostock	D1
Rothenburgsort	P
Rottnest	G
Rovaniemi	I
Royan	H
Ruanda	D1
Rubislaw	F
Ruiru	L
Rumford	C1
Runby	H
Ruzizi	E1
Saarbruecken	D1
Saboya	I
Sada	N
Saintemarie	52
Saintpaul	B
Salford	I
Saloniki	I
Samaru	S
Sambre	E4
Sandiego	B
Sandow	C2–C3
Sanga	C2–C3
Sangalkam	D2
Sangera	I
Sanjuan	C1
Sanktgeorg	M
Sanktjohann	G
Sanktmarx	E4
Santander	M
Santhiaba	R
Santiago	C2–C3
Sao	E4
Sapele	G
Saphra	I
Sara	H
Sarajane	B
Saugus	R
Schalkwijk	H
Schleissheim	B
Schoeneberg	E4
Schwabach	C1
Schwarzengrund	B
Schwerin	C2–C3
Sculcoates	I

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Seattle	M
Sedgwick	V
Seegefeld	E1
Sekondi	E1
Selby	M
Sendai	D1
Senegal	F
Senftenberg	E4
Senneville	N
Seremban	D1
Serrekunda	E1
Shamba	I
Shangani	E1
Shanghai	I
Shannon	E1
Sharon	F
Sheffield	P
Sherbrooke	I
Shikmonah	R
Shipley	C2–C3
Shomolu	M
Shoreditch	D2
Shubra	B
Sica	S
Simi	E1
Sinchew	E1
Sindelfingen	C2–C3
Singapore	C1
Sinstorf	E1
Sinthia	K
Sipane	T
Skansen	C2–C3
Slade	E4
Sljeme	X
Sloterdijk	B
Soahanina	H
Soerenga	N
Sokode	D2
Solna	M
Solt	F
Somone	C1
Soumbedioune	M
Southampton	B
Southbank	E1
Souza	E1
Spalentor	T
Spartel	L
Splott	V
Stachus	P
Stanley	B
Stanleyville	B
Staoueli	X
Steinplatz	N
Steinwerder	54
Stellingen	X
Stendal	F
Sternschanze	N
Sterrenbos	C2–C3
Stockholm	E1
Stoneferry	N
Stormont	E1
Stourbridge	C2–C3
Straengnaes	F
Strasbourg	D2
Stratford	E4
Strathcona	C1
Stuivenberg	E4
Stuttgart	C1
Suberu	E1
Sudan	U
Suelldorf	W
Sundsvall	H
Sunnycove	C2–C3
Surat	H
Surrey	L
Svedvi	E4
Sya	X
Sylvania	H
Szentes	I
Tabligbo	X
Tado	C2–C3
Tafo	B
Taiping	G
Takoradi	C2–C3
Taksony	E4
Tallahassee	C2–C3
Tamale	C2–C3
Tambacounda	E4
Tamberma	X
Tamilnadu	C1
Tampico	C1
Tananarive	C2–C3
Tanger	G
Tanzania	G
Tarshyne	D1
Taset	T
Taunton	M
Taylor	P
Tchad	O
Tchamba	J
Techimani	M
Teddington	B
Tees	I
Tejas	B
Teko	H
Telaviv	M
Telelkebir	G
Telhashomer	F
Teltow	M
Tema	T
Tendeba	J
Tennenlohe	K
Tennessee	C1
Tennyson	B
Teshie	X
Texas	B
Thayngen	B
Thetford	U
Thiaroye	P
Thies	E4
Thompson	C1
Tibati	E1
Tienba	C1
Tiergarten	V
Tiko	R
Tilburg	E4
Tilene	R
Tinda	B
Tione	51
Togba	I
Togo	B
Tokoin	B
Tomegbe	T
Tomelilla	E4
Tonev	54
Toowong	F
Torhout	N
Toricada	T
Tornow	W
Toronto	D2
Toucra	Y
Toulon	K

(*continued*)

TABLE BXII.γ.263. *(cont.)*

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Tounouma	C2–C3
Tours	F
Trachau	B
Transvaal	W
Travis	B
Treforest	51
Treguier	D1
Trier	I
Trimdon	D2
Tripoli	B
Trotha	R
Truro	E1
Tschangu	G
Tsevie	B
Tshiongwe	C2–C3
Tucson	H
Tudu	B
Tumodi	B
Tunis	G
Typeb	O
Typhi	D1
Typhimurium	B
Typhisuis	C1
Tyresoe	B
Uccle	54
Uganda	E1
Ughelli	E1
Uhlenhorst	V
Uithof	52
Ullevi	G
Umbilo	M
Umhlali	C1
Umhlatazana	O
Uno	C2–C3
Uppsala	B
Urbana	N
Ursenbach	T
Usumbura	K
Utah	C2–C3
Utrecht	52
Uzaramo	H
Vaertan	G
Valdosta	C2–C3
Vancouver	I
Vanier	M
Vaugirard	S
Vegesack	I
Vejle	E1
Vellore	B
Veneziana	F
Verona	S
Verviers	W
Victoria	D1
Victoriaborg	J
Vietnam	S
Vilvoorde	E4
Vinohrady	M
Virchow	C1
Virginia	C2–C3
Visby	E4
Vitkin	M
Vleuten	V
Vogan	T
Volkmarsdorf	M
Volta	F
Vom	B
Voulte	U
Vridi	G
Vuadens	B
Wa	I
Waedenswil	D2

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Wagadugu	E1
Wagenia	B
Wandsworth	Q
Wangata	D1
Waral	T
Warengo	J
Warmsen	W
Warnemuende	M
Warnow	C2–C3
Warragul	H
Warri	J
Washington	G
Waycross	S
Wayne	N
Wedding	M
Welikade	I
Weltevreden	E1
Wenatchee	X
Wentworth	F
Wernigerode	D2
Weslaco	T
Westafrica	D1
Westeinde	I
Westerstede	E4
Westhampton	E1
Westminster	E1
Weston	I
Westphalia	O
Weybridge	E1
Wichita	G
Widemarsh	O
Wien	B
Wil	C1
Wilhelmsburg	B
Willemstad	G
Wilmington	E1
Wimborne	E1
Windermere	Q
Wingrove	C2–C3
Winneba	B
Winnipeg	54
Winston	C1
Winterthur	E4
Wippra	C2–C3
Wisbech	I
Wohlen	F
Woodhull	H
Woodinville	F
Worb	D2
Worthington	G
Wuiti	N
Wuppertal	D2
Wyldegreen	G
Yaba	E1
Yalding	E4
Yaounde	B
Yardley	M
Yarm	C2–C3
Yarrabah	G
Yeerongpilly	E1
Yehuda	F
Yekepa	R
Yerba	54
Yoff	P
Yokoe	C2–C3
Yolo	O
Yopougon	W
Yoruba	I
Yovokome	C2–C3
Yundum	E1
Zadar	D2

(*continued*)

TABLE BXII.γ.263. *(cont.)*

Serovar	O Group
Zaiman	D1
Zaire	N
Zanzibar	E1
Zaria	J
Zega	D1
Zehlendorf	N
Zerifin	C2–C3
Zigong	I
Zinder	V
Zongo	E1
Zuilen	E4
Zwickau	I

animals; since the last decade, very frequent agent of *Salmonella* gastroenteritis in humans.

The mol% G + C of the DNA is: see species description.

Deposited strain: ATCC 13076.

iii. **Salmonella enterica** *subsp.* **enterica** *serovar* **Gallinarum** (Klein 1889) Bergey, Harrison, Breed, Hammer and Huntoon 1925, 236 (*Bacillus gallinarum* Klein 1889, 689; *Bacterium pullorum* Rettger 1909, 123; *Salmonella gallinarum-pullorum* Taylor, Bensted, Boyd, Carpenter, Dowson, Lovell, Taylor, Thornton, Wilson and Shaw 1952, 140.)
gal.li.na'rum. L. n. *gallina* hen; L. gen. pl. n. *gallinarum* of hens.

Antigenic formula: 1,9,12:−:−. The presence of factor O1 is connected with lysogenization. Always nonmotile. May be subdivided into biovars based on fermentation characteristics, production of gas, and production of H_2S. Does not grow on a minimal defined medium. Isolated chiefly from chickens and other birds. Causative agent of fowl typhoid.

The mol% G + C of the DNA is: see species description.

iv. **Salmonella enterica** *subsp.* **enterica** *serovar* **Paratyphi A** (Brion and Kayser 1902) Castellani and Chalmers 1919, 939 (*Bacterium paratyphi* Kayser 1902, 426; *Bacterium paratyphi* typhus A Brion and Kayser 1902, 613.)
pa.ra.ty'phi. Gr. prep. *para* alongside of; Gr. n. *typhus* a stupor; M.L. gen. n. *paratyphi A* of type A typhoid-like infection.

Antigenic formula: 1,2,12:a:−. The presence of factor O1 is connected with lysogenization. Aerogenic. Ferments arabinose but no xylose. The majority of strains do not produce H_2S, and in this respect serovar Paratyphi A is unlike most other salmonellae. Lysine decarboxylase reaction is weak or negative. Pathogenic only for humans.

The mol% G + C of the DNA is: see species description.

v. **Salmonella enterica** *subsp.* **enterica** *serovar* **Paratyphi B** (Brion and Kayser 1902) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 213 (*Bacterium paratyphi* typhus B Brion and Kayser 1902, 613; *Bacillus schottmuelleri* Winslow, Kligler and Rothberg 1919, 479.)
pa.ra.ty'phi. Gr. prep. *para* alongside of; Gr. n. *typhus* a stupor; M.L. gen. n. *paratyphi B* of type B typhoid-like infection.

Antigenic formula; 1,4,[5],12:b:1,2. The presence of factor O1 is connected with lysogenization. Produces a slime layer when grown on a medium containing 0.5% glucose and 0.2 M sodium phosphate, pH 7. Negative for D-tartrate. Causes enteric fever in humans and very rarely infects animals. A variant known as biovar Java is positive for D-tartrate, fails to produce a slime layer, and usually causes enteritis in humans and not uncommonly in animals as well (Kauffmann, 1941). Some strains are intermediate between these two extremes.

The mol% G + C of the DNA is: see species description.

vi. **Salmonella enterica** *subsp.* **enterica** *serovar* **Paratyphi C** Hirschfeld 1919, 296 (Paratyphoid C bacillus Hirschfeld 1919, 296; *Salmonella hirschfeldii* Weldin 1927, 161; *Salmonella* paratyphi-C International Salmonella Subcommittee 1934.)
pa.ra.ty'phi. Gr. prep. *para* alongside of; Gr. n. *typhus* a stupor; M.L. gen. n. *paratyphi C* of type C typhoid-like infection.

Antigenic formula; 6,7,[Vi]:c:1,5. Ferments dulcitol and trehalose; produces H_2S. Arabinose fermentation is variable.

The mol% G + C of the DNA is: see species description.

vii. **Salmonella enterica** *subsp.* **enterica** *serovar* **Typhi** (Schroeter 1886) Warren and Scott 1930, 416 (*Bacillus typhi* Schroeter 1886, 165.)
ty'phi. Gr. n. *typhus* a stupor; M.L. gen. n. *typhi* of typhoid.

Antigenic formula: 9,12,[Vi]:d:−. Wild strains may possess H antigen z66 instead of H antigen d (Guinée et al., 1981). Does not grow on Simmons' citrate medium or on a minimal defined medium; requires at least tryptophan as a growth factor. Does not produce gas from glucose or other sugars. Fermentation of xylose is variable. Many strains are agglutinated by anti-Vi serum and are inagglutinable by anti-09 serum; their colonies (V colonies) are opaque and have an iridescent appearance when examined by transmitted light. Colonies of intermediate appearance agglutinable by both Vi and O antisera may occur (VW colonies). Pathogenic only for humans, causing typhoid (enteric) fever; transmitted by water or food contaminated by human excreta.

The mol% G + C of the DNA is: see species description.

Deposited strain: ATCC 19430.

viii. **Salmonella enterica** *subsp.* **enterica** *serovar* **Typhimurium** (Loeffler 1892) Castellani and Chalmers 1919, 939 (*Bacterium typhimurium* Loeffler 1892, 134.)
ty.phi.mu'ri.um. Gr. n. *typhus* a stupor; L. n. *mus* mouse; L. gen. pl. n. *murium* of mice; M.L. gen. pl. n. *typhimurium* typhoid of mice.

Antigenic formula: 1,4,[5],12:i:1,2. The presence of factor O1 follows lysogenization by a converting phage named *iota* or PLT22. Ubiquitous and frequently the cause of infections in humans and animals; very frequent agent of *Salmonella* gastroenteritis in man.

The mol% G + C of the DNA is: see species description.

Deposited strain: ATCC 13311.

b. **Salmonella enterica** *subsp.* **arizonae** (Borman 1957) Le Minor and Popoff 1987, 467 (*Salmonella choleraesuis* subsp. *arizonae* (Borman 1957) Le Minor, Véron and Popoff 1985, 375; *Salmonella arizonae* (Borman 1957) Kauffmann *in* van Oye 1964; *Paracolobactrum arizonae* Borman 1957, 347.)
a.ri.zo′nae. M.L. gen. n. *arizonae* of Arizona, a state in the United States.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 94 serovars (Popoff et al., 1998). All serovars belonging to this subspecies are monophasic for the H antigen. Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 51:z_4,z_{23}:−.
The mol% G + C of the DNA is: see species description.
Type strain: ATCC 13314, CIP 82.30, NCTC 8297.

c. **Salmonella enterica** *subsp.* **diarizonae** (Le Minor, Véron and Popoff 1985) Le Minor and Popoff 1987, 467 (*Salmonella choleraesuis* subsp. *diarizonae* Le Minor, Véron and Popoff 1985, 375.)
di.a.ri.zo′nae. Gr. adj. *dis* twice, two; M.L. gen. n. *arizonae* of Arizona, a state in the United States.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 323 serovars (Popoff et al., 1998). All serovars belonging to this subspecies are diphasic for the H antigen. Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 6,7:l,v:z_{53}.
The mol% G + C of the DNA is: see species description.
Type strain: ATCC 43973, CIP 82.31, NCTC 10060.

d. **Salmonella enterica** *subsp.* **houtenae** (Kauffmann 1962) Le Minor and Popoff 1987, 467 (*Salmonella choleraesuis* subsp. *houtenae* (Kauffmann 1962) Le Minor, Rohde and Taylor 1970b, 209; *Salmonella houtenae* Kauffmann 1962, 353.)
hou′te.nae. M.L. gen. n. *houtenae* of Houten, a town in Holland.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 70 serovars (Popoff et al., 1998). All serovars belonging to this subspecies are monophasic for the H antigen. Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 41:g,z_{51}:−.
The mol% G + C of the DNA is: see species description.
Type strain: ATCC 43974, CIP 82.32, NCTC 10401.

e. **Salmonella enterica** *subsp.* **indica** (Le Minor, Popoff, Laurent and Hermant 1987) Le Minor and Popoff 1987, 467 (*Salmonella choleraesuis* subsp. *indica* Le Minor, Popoff, Laurent and Hermant 1987, 179.)
in.di′ca. L. adj. *indica* of India.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 11 serovars (Popoff et al., 1998). Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 1,6,14,25:a:e,n,x.
The mol% G + C of the DNA is: see species description.
Type strain: ATCC 43976, CIP 102501.

f. **Salmonella enterica** *subsp.* **salamae** (Le Minor, Rohde and Taylor 1970b) Le Minor and Popoff 1987, 467 (*Salmonella choleraesuis* subsp. *salamae* (Le Minor, Rohde and Taylor 1970b) Le Minor, Véron and Popoff 1985, 375) (*"Salmonella salamae"* Le Minor, Rohde and Taylor 1970b, 209.)
sa.la′mae. M.L. gen. n. *salamae* of (Dare-es) salaam.

Distinguished from other *S. enterica* subspecies by the characteristics reported in Table BXII.γ.261. Contains at least 488 serovars (Popoff et al., 1998). Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 1,9,12:l,w:e,n,x.
The mol% G + C of the DNA is: see species description.
Type strain: ATCC 43972, CIP 82.29, NCTC 5773.

2. **Salmonella bongori** (Le Minor, Véron and Popoff 1985); Reeves, Evins, Heiba, Plikaytis and Farmer 1989b, 371[VP] (Effective publication: Reeves, Evins, Heiba, Plikaytis and Farmer 1989a, 319) (*Salmonella choleraesuis* subsp. *bongori* Le Minor, Véron and Popoff 1985, 375.)
bon′gori. M.L. gen. n. *bongori* of Bongor, a town in Chad.

The characteristics are as described for the genus (see also Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*) and as listed in Table BXII.γ.261. All serovars belonging to *S. bongori* are monophasic for the H antigen. Contains at least 20 serovars (Popoff et al., 1998). Isolated mainly from cold-blooded animals and environment.

Antigenic formula: 66:z_{41}:−.
The mol% G + C of the DNA is: 51.8 ± 0.6 (T_m).
Type strain: ATCC 43975, CIP 82.33.
GenBank accession number (16S rRNA): AF029227.

Genus XXXIV. **Serratia** *Bizio 1823, 288*[AL]

FRANCINE GRIMONT AND PATRICK A.D. GRIMONT

Ser.ra′ti.a. M.L. fem. n. *Serratia* named after Serafino Serrati, an Italian physicist.

Straight rods, 0.5–0.8 × 0.9–2.0 μm in length, with rounded ends. Conform to the general definition of the family *Enterobacteriaceae*. Gram negative, generally **motile**, by means of peritrichous flagella. **Facultatively anaerobic. Nitrate and chlorate are reduced anaerobically. Growth factors are generally not required.** Colonies on nutrient agar are most often opaque, somewhat iridescent, and either **white, pink, or red in color**. Almost all strains can grow at temperatures between 10 and 36°C, at pH 5–9, and in the presence of 0–4% (w/v) NaCl. The catalase reaction is strongly positive. **D-Glucose is fermented** through the Embden–Meyerhof pathway. The major glucose entry route involves a phosphoenolpyruvate-dependent phosphotransferase system with both enzyme II^{Glc} (glucose permease) and enzyme II^{Man} (mannose permease). Glucose is also oxidized to gluconate in the presence of pyrroloquinoline quinone. **Gluconate is oxidized to 2-ketogluconate. Acetoin is produced from pyruvate** by all species except *S. fonticola*. Fructose, D-galactose, maltose, D-mannitol, D-mannose, ribose, and trehalose are fermented and utilized as sole carbon sources. L-fucose is fermented and utilized as sole carbon source by all species except *S. fonticola*. L-sorbose

is not fermented or utilized as sole carbon source. All species but *S. fonticola* fail to ferment or utilize dulcitol and tagatose. *N*-acetylglucosamine, D-alanine, L-alanine, citrate, D-galacturonate, D-glucosamine, D-glucuronate, 2-ketogluconate, L-proline, putrescine, L-serine are utilized as sole carbon sources by most strains. Caprate, caproate, **caprylate**, and tyrosine are **utilized** as sole carbon sources by all species except *S. fonticola*. 5-Aminovalerate, butyrate, *m*-coumarate, ethanolamine and tryptamine are not utilized as sole carbon sources. All species except *S. fonticola* fail to utilize 3-phenylpropionate. All species except *S. entomophila* fail to utilize itaconate. Phenylalanine, histidine, and tryptophan deaminases and thiosulfate reductase (H_2S from thiosulfate) are not produced. ***o*-Nitrophenyl-β-D-galactopyranoside** (ONPG) is **hydrolyzed by most strains**. Esculin is hydrolyzed by most strains except *S. proteamaculans* subsp. *quinovora*. **Extracellular enzymes** of all species except *S. fonticola* **hydrolyze DNA, lipids** (tributyrin, corn oil) **and proteins** (gelatin, casein), but not starch (in four days), polygalacturonic acid, or pectin. Tween-80 is hydrolyzed by all species except *S. odorifera*. The organisms **occur in the natural environment** (soil, water, plant surfaces) or **as opportunistic human pathogens**.

The mol% G + C of the DNA is: 52–60.

Type species: **Serratia marcescens** Bizio 1823, 288.

Further descriptive information

Phylogenetic affinities among *Serratia* species have been studied (Fig. BXII.γ.208) (Dauga et al., 1990; Spröer et al., 1999). Three clusters can be observed in all studies: cluster I includes *S. marcescens* and *S. rubidaea*, cluster II includes psychrotrophic species (*S. proteamaculans*, *S. grimesii*, *S. liquefaciens*, *S. plymuthica*, and *S. fonticola*), and cluster III includes *S. ficaria* and *S. entomophila*. Depending on the analysis, *S. odorifera* may branch with cluster I or after clusters II and III have merged. Sequence comparisons leave no doubt as to the affiliation of *S. fonticola* to the genus *Serratia* and especially to the psychrophilic group of species.

Cells of *Serratia* rarely show a visible capsule in India ink mounts, although mucoid colonies can be observed in *S. plymuthica* and occasionally in other *Serratia* species; however, cells of *S. odorifera* possess a microcapsule which can be evidenced by the quellung reaction (capsular swelling) using *Klebsiella* anticapsule K4 or K68 sera (Richard, 1979). Polysaccharides excreted by cells of *S. marcescens* can be extracted from the cell surface layer or from the culture medium. These polysaccharides contain chiefly D-glucose and glucuronic acid and lower proportions of D-mannose, heptose, L-fucose and L-rhamnose (Adams and Martin, 1964; Adams and Young, 1965).

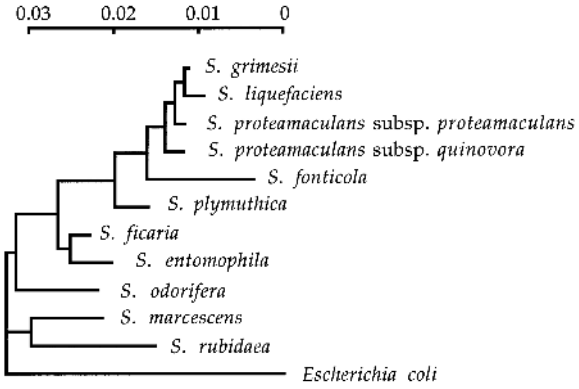


FIGURE BXII.γ.208. Phylogenetic tree of the genus *Serratia*. The tree was built by the neighbor-joining method (Saitou and Nei, 1987). Scale is in K_{nuc} according to Jukes and Cantor (1969).

The major fatty acid components found in whole-cell methanolysates are $C_{14:0\ 3OH}$, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$, contributing 50–80% of the components in each strain. $C_{14:0}$ contributes 3.7–9.4% whereas other components contribute less than 3% each (Bergan et al., 1983).

Colony diameters are ~1.5–2.0 mm after overnight growth on nutrient agar. Swarming does not occur.

Two different pigments can be produced by various *Serratia* strains: prodigiosin and pyrimine (Williams and Qadri, 1980). Prodigiosin, a nondiffusible, water-insoluble, red pigment bound to the cell envelope, is produced by two biogroups (A1 and A2) of *S. marcescens* and by most strains of *S. plymuthica* and *S. rubidaea*. Prodigiosin-producing colonies are totally red or show either a red center, a red margin, or red sectors. The exact color given by the pigment depends upon cultural conditions (e.g., amino acids, carbohydrates, pH, inorganic ions, temperature) and may include orange, pink, red, or magenta. Prodigiosin is best produced on peptone-glycerol agar at 20–35°C. The temperature range for pigment production is 12–36°C. Prodigiosin is not produced anaerobically. Chemically, prodigiosin is a tripyrrole derivative, 2-methyl-3-amyl-6-methoxyprodigiosene (prodigiosene is 5-(2-pyrryl)-2,2′-dipyrrylmethene). In the cell, prodigiosin is formed by condensation of a volatile 2-methyl-3-amylpyrrol (MAP) and a nonvolatile 4-methoxy-2-2′-bipyrrole-5-carboxaldehyde (MBC). Several classes of nonpigmented mutants that are blocked on either the MAP pathway or the MBC pathway have been isolated. Syntrophic pigmentation may occur when two different class mutants are grown side by side (Williams and Qadri, 1980).

Pyrimine, a water-soluble, diffusible pink pigment (Williams and Qadri, 1980), is produced by some strains of *S. marcescens* biogroup A4. Ferrous iron is required for the production of pyrimine. Pyrimine is L-2(2-pyridyl)-D′-pyrroline-5-carboxylic acid. When pyrimine is produced, the agar medium turns pink while the colonies are white to pinkish.

A yellow diffusible pigment, 2-hydroxy-5-carboxymethylmuconic acid semialdehyde, is produced from the meta cleavage of 3,4-dihydroxyphenylacetic acid (3,4-DHP) by the enzyme 3,4-DHP 2,3-dioxygenase (Trias et al., 1988), induced by tyrosine in all *S. marcescens* strains. At present, only *S. marcescens* strains of biotype A8a which have lost the ability to grow on aromatic compounds can produce the yellow pigment. A new reddish violet pigment, a peptide-ferropyrimine complex, produced by a *S. marcescens* O5:H1 was described by Suzuki et al. (1993b).

Cultures can produce two kinds of odors, a fishy to urinary odor attributed to trimethylamine (mixed with some NH_3), or a musty, potato-like odor attributed to alkyl-methoxypyrazine. The musty odor produced by *S. odorifera*, *S. ficaria*, and a few strains of *S. rubidaea* is due to 3-isopropyl-2-methoxy-5-methylpyrasine (Gallois and Grimont, 1985). All other strains and species produce the fishy-urinary odor.

All species except *S. marcescens* and *S. rubidaea* can grow readily at 4–5°C and several grow at 40°C (*S. marcescens* and several strains of *S. rubidaea* and *S. odorifera*); however, the temperature of 37°C is not favorable for the isolation of *S. plymuthica*. When *S. liquefaciens* and *S. plymuthica* are studied, many tests that are positive at 28–35°C give negative results at 37°C (e.g., Voges–Proskauer, decarboxylases, tetrathionate reductase tests).

A strong catalase activity, which can be evidenced with 3% (or

less) H_2O_2, is produced by *Serratia* strains (Taylor and Achanzar, 1972).

There is no sodium ion requirement for growth in the genus *Serratia*; however, the optimum concentration of NaCl for growth is ~0.5% (w/v) for *S. marcescens* or 1% (w/v) for *S. rubidaea* (Grimont and Grimont, unpublished results). Tolerance to NaCl ranges from 5 to 6% (w/v) for *S. plymuthica*, to 10% (w/v) for *S. rubidaea*.

In Biotype-100 strips (BioMerieux, Craponne, France) or a minimal medium containing ammonium sulfate as the nitrogen source, the following compounds serve universally as sole carbon sources for all *Serratia* strains: *N*-acetylglucosamine, *cis*-aconitate, citrate, D-fructose, D-galactose, D-galacturonate, D-gluconate, D-glucosamine, D-glucose, *myo*-inositol, 2-ketogluconate, maltose, maltotriose, D-mannitol, D-mannose, D-ribose, and D-trehalose. Most strains of all species can utilize D-alanine, L-alanine, D-glucuronate, glycerate, glycerol, lactate, putrescine, L-serine, and L-tryptophan. All species except some or all strains of *S. fonticola* can utilize L-aspartate, L-fucose, fumarate, L-glutamate, L-malate, L-proline, and succinate. Utilization of caprate, caproate, and caprylate by all species except *S. fonticola* is better observed in a minimal agar containing ammonium sulfate as the nitrogen source, rather than in Biotype-100 strips. The following compounds are never utilized as sole carbon sources (Biotype-100 strips): DL-5-aminovalerate, *m*-coumarate, ethanolamine, glutarate, sorbose, and tryptamine. The following carbon sources are never utilized by *Serratia* species except some or all *S. fonticola* strains: dulcitol, 3-phenylpropionate, and tagatose (Grimont et al., 1977b, 1978b, 1979b; Grimont and Grimont, 1978b).

Characteristic extracellular enzymes are produced by most species. All species except *S. fonticola* can hydrolyze DNA, gelatin, soluble casein, tributyrin, and corn oil. Only rare strains fail to produce one or more of these extracellular enzymes. All species, except *S. odorifera*, can hydrolyze Tween 80. Chitin is hydrolyzed by all species except *S. rubidaea*, *S. odorifera*, and *S. fonticola*. Lecithin is also hydrolyzed by many strains. Spot-inoculated starch agar (Starch agar: nutrient agar containing 0.5% [w/v] soluble starch), incubated for four days and then flooded with Lugol's iodine, shows no zone of clearing (Grimont et al., 1977b); however, longer incubation (6–14 days) may allow detection of some amylase-producing strains (M. Popoff, personal communication).

A red-pigmented *S. marcescens* has been found to produce a carboxymethyl cellulase (Thayer, 1978). Depolymerization of a carboxymethylcellulose gel is faster with *S. marcescens*, *S. rubidaea*, and *S. liquefaciens* than with *S. odorifera*, *S. ficaria*, and *S. plymuthica* (unpublished results).

Up to 11 proteinases have been revealed by agar gel electrophoresis. Each strain produces one to four different proteinases. Different species have different proteinase patterns (Grimont et al., 1977a). These have been used to type strains (Grimont and Grimont, 1978c). Isoelectric points of the 11 proteinases are between pH 3.6 and pH 6.0 (Grimont and Grimont, 1978b).

Fructose, maltose, D-mannitol, D-mannose, ribose, and trehalose are fermented by all strains. Most strains ferment glycerol and *myo*-inositol. Fermentation of D-glucose is not prevented by 0.001 M iodoacetate (Grimont et al., 1977b, 1978b, 1979b; Grimont and Grimont, 1978b), an inhibitor of the Embden–Meyerhof–Parnas glycolytic pathway and other enzymic reactions. *Serratia* species can produce gluconate-6-phosphate dehydrase and 2-keto-3-deoxygluconate-6-phosphate aldolase (Kersters and De Ley, 1968), which are the characteristic enzymes of the Entner–Doudoroff pathway.

Under aerobic conditions, gluconate is produced from D-glucose in the presence of iodoacetate by all *Serratia* species due to a glucose dehydrogenase, when pyrroloquinoline quinone (PQQ) is added. Only *S. marcescens*, *S. odorifera*, *S. rubidaea*, *S. entomophila*, *S. ficaria*, and some strains of *S. plymuthica* can produce gluconate without added PQQ (Bouvet et al., 1989).

A reducing compound, 2-ketogluconate, is also produced from gluconate by all species due to a gluconate dehydrogenase (Grimont et al., 1977b, 1978a, 1979b; Bouvet et al., 1989).

2,5-Diketogluconate is produced from 2-ketogluconate at 20°C (not at 30°C) due to a 2-ketogluconate dehydrogenase by *S. marcescens*, *S. liquefaciens*, and *S. grimesii* (Bouvet et al., 1989).

In anaerobic glycerol dissimilation, a glycerol dehydrogenase induced by glycerol and not by hydroxyacetone is present in *S. marcescens*, *S. proteamaculans*, *S. liquefaciens*, *S. grimesii*, and *S. fonticola*, but not in *S. rubidaea*, *S. ficaria*, *S. odorifera*, *S. entomophila*, and *S. plymuthica* (Bouvet et al., 1995a). The Voges–Proskauer (VP) test, when done on a 3-day-old culture in Clark–Lubs medium, is negative for 40% of strains of *S. plymuthica*, although acetoin can be detected after incubation for 18 h by use of a sensitive method (Richard, 1972). These strains, which are VP-negative after 3 days of incubation, can utilize 2,3-butanediol as a sole carbon source (Grimont et al., 1978b). *Serratia* strains, other than *S. fonticola*, that cannot produce acetoin from pyruvate (under any experimental conditions) are very rare. *S. fonticola* is always negative for acetoin. A tiny gas bubble is commonly produced by *S. marcescens* in a peptone–water–glucose medium with Durham tube. *S. plymuthica* and *S. liquefaciens* produce a large amount of gas. The end products of glucose fermentation by *S. marcescens* are 2,3-butanediol, ethanol, formate, lactate, succinate, and CO_2 with small amounts of acetate, acetoin, and glycerol and very little or no H_2 (Neish et al., 1948; White and Starr, 1971). The end products yielded by *S. plymuthica* are 2,3-butanediol, ethanol, lactate, succinate, CO_2, H_2, and small amounts of formate, acetate, acetoin, and glycerol (Neish et al., 1948). The 2,3-butanediol produced by *S. marcescens* is mostly a *meso*-isomer, whereas *S. plymuthica* is unique in producing a levo-rotatory 2,3-butanediol (Neish et al., 1948).

Transduction systems in *S. marcescens* have been described (Kaplan and Brendel, 1969; Matsumoto et al., 1973). The earliest genetic transfer described in *S. marcescens* (Belser and Bunting, 1956) is also suggestive of a transduction mechanism. *S. marcescens* was transformed with plasmid pBR322 by Reid et al. (1982). Transformants were selected based on resistance to high levels of ampicillin.

All species of the genus *Serratia* have been delineated by DNA–DNA hybridization. All species of the genus except *S. fonticola* share a number of phenotypic properties. *S. fonticola* was included in the genus *Serratia* because of significant DNA relatedness with other *Serratia* species (Steigerwalt et al., 1976; Gavini et al., 1979); this inclusion was confirmed by comparison of 16S rDNA gene sequences (Dauga et al., 1990). A summary of DNA relatedness within the genus *Serratia* is given in Table BXII.γ.264.

Lactose plasmids have been demonstrated in *S. liquefaciens* (Le Minor et al., 1974) and in *S. marcescens* (C. Coynault, personal communication). Antibiotic resistance plasmids of incompatibility groups S (= H_2), C, L/M, P, W, and FII have been identified in *S. marcescens*. Plasmids of groups M and N have been found in *S. liquefaciens* (Hedges, 1980). Replicon typing, using cloned DNA probes, identified plasmid groups FIB, FIC, FIIA, H12, L/M, N, B/O, P, W, Y, and Com9 in multiresistant *S. marcescens* strains (Llanes et al., 1994).

TABLE BXII.γ.264. DNA relatedness among *Serratia* species[a,b]

	Source of labeled DNA										
Species	*S. marcescens*[c]	*S. entomophila*	*S. fonticola*	*S. ficaria*[d]	*S. grimesii*	*S. liquefaciens*	*S. odorifera*[c]	*S. plymuthica*	*S. proteamaculans* subsp. *proteamaculans*	*S. proteamaculans* subsp. *quinovora*	*S. rubidaea*
S. marcescens	**92 ± 5.5** **d = 0–3.2**	42 ± 6.3 d = 7.5–8.0	50 ± 5.1	46 ± 4.7 d = 8.5–9.5	36	29	31 ± 2.8 d = 10.5–11.5	29	34	33	26 ± 2.1
S. entomophila	ND	**84 ± 8.2** **d = 0–1.0**	ND	62 ± 5.4 d = 6.0–9.0	ND	ND	ND	ND	ND	ND	ND
S. fonticola	57 ± 4.9 d = 12.0–14.0	28	**88 ± 5.5**	40 d = 10.0	22	23	38 ± 2.8 d = 13.0	21	29	22	16
S. ficaria	ND	47 ± 6.4 d = 5.5–8.5	ND	**91 ± 4.2** **d = 0–2.0**	44	38	ND	44	41	47 d = 10.0	27 ± 3.4
S. grimesii	53 ± 1.4 d = 12.0–12.5	34 ± 6.0	ND	29	**97 ± 6.4** **d = 0–4.0**	36 ± 3.8 d = 9.5–14.0	32 ± 1.1 d = 13.5	36 ± 4.6 d = 11.0–15.5	42 ± 5.5 d = 9.5–10.5	44 ± 3.4 d = 8.5–15.0	18 ± 2.6
S. liquefaciens	62 ± 1.1 d = 10.5–12.5	48	41 ± 2.1	33 ± 1.7	47 ± 7.0 d = 10.0–11.5	**86 ± 7.5** **d = 0–3.0**	39 ± 3.5 d = 11.5	44 ± 5.1 d = 8.5–12.5	45 ± 7.1 d = 7.5–9.5	48 ± 5.7 d = 8.0–9.5	19 ± 5.0
S. odorifera	ND	40	ND	33 ± 2.8 d = 16.5	29	26	**78 ± 13.9** **d = 0–4.0**	28	28	25	21 ± 4.2
S. plymuthica	62 d = 10.0	42	44 ± 5.7	43 ± 4.6 d = 13.0	43 ± 4.1 d = 9.5–12.0	39 ± 5.2 d = 8.5–10.0	38 ± 2.0 d = 11.0	**85 ± 14.6** **d = 0–4.5**	52 ± 2.3 d = 8.5–9.5	44 ± 4.7 d = 9.0–12.0	20 ± 2.5
S. proteamaculans subsp. *proteamaculans*	ND	40	ND	41 ± 5.6	52 ± 5.6 d = 9.0–13.0	42 ± 5.4 d = 9.0–13.0	ND	45 ± 5.7 d = 9.0–12.0	**81 ± 11.0** **d = 0–6.5**	64 ± 5.3 d = 5.5–12.0	21 ± 4.8
S. proteamaculans subsp. *quinovora*	ND	ND	ND	ND	52 ± 3.5 d = 8.5–10.0	44 ± 2.5 d = 8.5–10.5	ND	48 ± 4.4 d = 9.0–12.0	58 ± 7.4 d = 5.0–6.0	**88 ± 6.7** **d = 0–4.5**	ND
S. rubidaea	49 ± 1.7 d = 10.0–11.0	34	38 ± 18.7	26 ± 3.0 d = 12.0	24	24	32 ± 3.2 d = 10.0	26	26	30	**88 ± 20.6** **d = 0–6.0**

[a]All DNA relatedness values obtained with the S1 nuclease method except otherwise stated. Divergence (d) given rounded to the nearest 0.5.

[b]Data from Steigerwalt et al. (1976), Grimont et al. (1978a, 1979b, 1982b, 1988), and Gavini et al. (1979). Data in the *S. rubidaea* column are unpublished.

[c]DNA relatedness values obtained with the hydroxyapatite method.

[d]DNA relatedness values obtained with the filter method.

Bacteriophages active on *Serratia* are easily found in river water or sewage. Phages that are active on one species of *Serratia* are usually active on strains of other species of that genus, but rarely on strains of other genera (Grimont and Grimont, 1978b). Lysogeny is very common in *Serratia* species (Prinsloo, 1966). Several phage typing systems have been studied (Pillich et al., 1964; Hamilton and Brown, 1972; Farmer, 1974; Grimont, 1977a).

Bacteriocins produced by *Serratia* are of two kinds: (a) a trypsin-resistant, acid-sensitive (pH 2) structure (Hamon and Péron, 1961) called "group A bacteriocin" by Prinsloo (1966) and later found by electron microscopy to resemble phage tails (Traub, 1972); and (b) a trypsin-sensitive, acid-resistant protein (Hamon and Péron, 1961) called "group B bacteriocin" by Prinsloo (1966). Bacteriocins produced by one species of *Serratia* frequently cross-react with other species of this same genus. *Serratia* bacteriocins are also frequently active on *Escherichia coli* K12. *S. marcescens* strains produce group A and/or group B bacteriocins. *S. rubidaea* strains produce only group A bacteriocins. *S. liquefaciens* and *S. ficaria* produce only group B bacteriocins. *S. odorifera* produces neither group A nor group B bacteriocins (Hamon and Péron, 1979; Y. Hamon, personal communication). Bacteriocin typing can be used for epidemiological purposes (Traub, 1980).

The antigenic structure of *S. marcescens* has been described. The present scheme consists of 28 somatic antigens (O1 to O28) and 25 flagellar antigens (H1 to H25) (Edwards and Ewing, 1972; Le Minor and Pigache, 1978; Traub and Fukushima, 1979a, b; Le Minor and Sauvageot-Pigache, 1981; Traub, 1991; Aucken et al., 1996). Subdivision of antigens O5 (into O5a, O5b, O5c), O10 (into O10a, O10b), and O16 (into O16a, O16b, O16c, O16d) has been proposed (Le Minor and Sauvageot-Pigache, 1981). Cross-reactions between factors O6 and O14 are very extensive and the distinction between these two factors does not seem worthwhile. Serovar O27 cross-reacts with the O4 serovar strain, and serovar O28 with the O5 serovar strain. The O-typing scheme was improved by separating capsular material from O-antigen (Gaston and Pitt, 1989a, b). H antigens are monophasic in *S. marcescens* (Aucken et al., 1996).

Four serovars (O1:H1, O2:H1, O3:H1, and O4:H1), all sharing a common H antigen, were described among *S. ficaria* strains (Grimont and Deval, 1982).

Resistance to cephalothin, colistin, and polymyxin (with respect to achievable serum levels of antibiotics) is very frequent in the genus and almost constant in *S. marcescens*. With the antibiotic disk method, a zone phenomenon develops around disks impregnated with colistin and polymyxin: the inhibition zone contains colonies close to the disk. However, this zone phenomenon is not restricted only to *Serratia*. Resistance to tetracycline and ampicillin is very frequent in *S. marcescens* and rare in other *Serratia* species. Plasmid-determined resistance to aminoglycoside antibiotics, carbenicillin, chloramphenicol, trimethoprim, sulfonamides, and mercury ions can be found in clinical strains of *S. marcescens*. "Third generation" cephalosporins are still active on *S. marcescens* and in a multicenter survey in the USA, fewer than 8% of *S. marcescens* isolates were resistant to piperacillin, 3–4% were resistant to ceftazidime, ceftriaxone, and/or cefotaxime, and 0.3% were resistant to imipenem (Jones, 1998). Resistance to cetyl trimethylammonium chloride (1.5 mg/ml) and thallus acetate (0.8 mg/ml) is very frequent (Grimont et al., 1977b). Of all the *Serratia* species, *S. marcescens* is the most resistant to antibiotics, antiseptics, and metal ions; *S. plymuthica* is the least resistant to these antimicrobials.

Healthy humans are not often infected by *Serratia*. *S. marcescens* is a prominent opportunistic pathogen for hospitalized human patients. At present, *S. marcescens* is the only known nosocomial species of *Serratia*. Clinically, *Serratia* infections do not differ from infections by other opportunistic pathogens (von Graevenitz, 1980). Other *Serratia* species can be involved in respiratory tract infection or colonization and septicemia, especially when these bacteria are accidentally injected into the body (e.g., contaminated perfusion or irrigation liquid) (Grimont and Grimont, 1978b).

S. marcescens and *S. liquefaciens* are known to infect and cause mortality in a variety of insects which can be serious pests of crops, ornamentals, and turf throughout the world (Klein and Kaya, 1995). Commercial utilization of *S. entomophila* against the grass grub *Costelytra zealandica* in New Zealand pastures has been achieved (Klein and Jackson, 1992). This bacterium turns the grubs a honey or amber color. Pathogenic strains of *S. entomophila* colonize the larva gut, adhere to the crop, and induce starvation, which causes depletion of the fat bodies. Pathogenicity is correlated with the production of lecithinase, proteinase, and chitinase (Kaska, 1976; Lysenko, 1976).

Mastitis in cows and other animal infections have been associated with *Serratia* species (Grimont and Grimont, 1978b). Pathogenicity in experimental animals is of the type expected of a Gram-negative bacterium. Experimental depression of phagocytic cell number or function in animals enhances susceptibility to *Serratia* infections (Simberkoff, 1980).

A typical hypersensitivity reaction is produced by inoculation of plants such as tobacco and king protea with *Serratia* (Lakso and Starr, 1970; Grimont et al. 1978b). *S. proteamaculans* was isolated from a leaf spot disease of *Protea cynaroïdes* (Paine and Stanfield, 1919) and *S. marcescens* (under the name *Erwinia amylovora* var. *alfalfae*) was isolated from a root disease of alfalfa (Shinde and Lukezic, 1974a).

Serratia species occur on plants, in the digestive tract of rodents (unpublished data), and in soil and water. *S. ficaria* is especially associated with the fig/fig-wasp ecosystem (Grimont et al., 1979b).

Enrichment and Isolation Procedures

Fecal samples (diluted with distilled water) or plant material washings are inoculated onto caprylate–thallus (CT) agar (Starr et al., 1976). After 2–5 d, the growth is removed by scraping and tested for deoxyribonuclease (DNase) activity. DNase-positive cultures are then purified by streaking on a nonselective medium (e.g., tryptic soy agar). Different colonial types are tested for DNase, and DNase-positive isolates are then thoroughly characterized and identified This procedure allows isolation of all *Serratia* species as defined in this chapter. *Providencia*, *Acinetobacter*, and fluorescent *Pseudomonas* strains can grow on CT agar when samples contain large numbers of these organisms. Other selective media based on DNase production and antibiotic resistance have been proposed (Cate, 1972; Berkowitz and Lee, 1973; Farmer et al., 1973). These antibiotic-containing media are efficient for the isolation of *S. marcescens* but may not be as reliable for more sensitive species (e.g., *S. plymuthica*).

Maintenance Procedures

For short-term preservation (several months), heavy suspensions of bacteria in sterile distilled water are made from bacterial growth scraped with a platinum loop from a nutrient agar slant. The suspensions are stored at room temperature. For longer preservation (several years), screw-capped tubes containing semi-

solid nutrient agar are stab-inoculated. After overnight growth at 30°C, the tubes are tightly closed and kept at room temperature in the dark. Maintenance failure may occur if the tube is not protected from desiccation by a rubber seal in the screw cap. Rubber corks dipped in melted paraffin wax may be preferred in place of screw caps.

Bacterial suspensions in brain–heart infusion supplemented with 50% glycerol and glass beads can be frozen at −80°C (cryoconservation). When needed, a glass bead can be taken without thawing the cryoconservation and transferred to a sterile broth for subculture. For long-term preservation (over 5 years), freeze-drying is preferred.

Procedures for Testing Special Characters

Carbon source utilization test This test is done by using Biotype-100 strips (bioMérieux, Craponne, France) that contains 99 pure carbon sources. Bottles of Biotype medium 1 are inoculated with a calibrated bacterial suspension (Grimont et al., 1996). The strips are incubated at 30°C for 4 d. Growth is scored visually after 2 and 4 days by comparison with the control cupule (without carbon source). The incubation day when growth is observed (1–4) is recorded for each cupule. Program Recognizer (Taxotron package, Institut Pasteur, Paris, France) can be used to enter the Biotype-100 data in an Apple Macintosh for automatic identification (Grimont et al., 1996).

Glucose oxidation test A 1-liter portion of glucose oxidation medium is composed of basal medium containing 8 g of nutrient broth (Difco Laboratories, Detroit, Mich.), 0.02 g of bromcresol purple, and 0.62 g of $MgSO_4 \cdot 7H_2O$ (2.5 mM). To 9.2 ml of autoclaved (121°C, 15 min) basal medium is added 0.4 ml of a filter-sterilized 1 M D-glucose solution (final concentration, 40 mM). This medium is supplemented with 0.4 ml of a fresh, sterile 25 mM iodoacetate solution (final concentration, 1 mM). The glucose oxidation medium is distributed (0.5–ml portions) into glass tubes (11 by 75 mm), which are plugged with sterile cotton wool. Bacteria grown overnight at 20 or 30°C on tryptocasein soy agar (Biorad, Marnes-la Coquette, France) supplemented with 0.2% (w/v) D-glucose are collected with a platinum loop, suspended in a sterile 2.5 mM $MgSO_4$ solution and adjusted to an absorbance at 600 nm of about 4. Glucose oxidation medium is then inoculated with 50 μl of bacterial suspension and vigorously shaken at 270 strokes per minute overnight at 20 or 30°C. The test is positive when a yellow color develops (acid production). In a negative test, the medium remains purple. When negative, the glucose oxidation test is repeated in the presence of 10 μM pyrroloquinoline quinone (PQQ). The control strains used are the type strains of *S. marcescens* (positive without requirement for PQQ), *S. liquefaciens* (positive only when PQQ is supplied), and *Hafnia alvei* (negative) (Bouvet et al., 1989).

Gluconate- and 2-ketogluconate dehydrogenase tests The gluconate dehydrogenase test is done as follows (Bouvet et al., 1989). Bacteria are grown overnight at 20°C on tryptocasein soy agar supplemented with 0.2% D-gluconate, then collected with a platinum loop and suspended in sterile distilled water to an absorbance (at 600 nm) of about 4. The reaction medium contains 0.2 M acetate buffer (pH 5), 1% (w/v) Triton X-100, 2.5 mM $MgSO_4$, 75 mM gluconate and (added immediately before use) 1 mM iodoacetate. A control medium contains the same ingredients except gluconate. The reaction medium and the control medium are dispensed (100-μl portions) into 96-well microtiter plates (Dynatech AG, Denkendorf, FRG). Bacterial suspensions (10 μl) are added to the reaction and control media and the microtiter plates are incubated at 20°C for 20 min. Then, 10-μl portions of a 0.1 M potassium ferricyanide solution (kept in the dark at room temperature for no longer than 1 week) are added to the wells and the plates are gently shaken and incubated at 20°C for 40 min. Then 50-μl portions of a reagent ($Fe_2(SO_4)_3$, 0.6 g; SDS, 0.36 g; 85% phosphoric acid, 11.4 ml; distilled water to 100 ml) are added to the wells. The plates are examined for the development of a green to blue color (due to Prussian blue) within 15 min at room temperature. The color in the uninoculated control medium remains yellow. The suggested control strains used are *Escherichia coli* K12 (negative) and the type strain of *Serratia marcescens* (positive).

The 2-ketogluconate dehydrogenase test is the same as above, except that the control and reaction media are adjusted to pH 4.0 and 2-ketogluconate is used in place of gluconate in the reaction medium. Suggested control strains are *Escherichia coli* K12 (negative) and the type strain of *Serratia marcescens* (positive at 20°C, not at 30°C) (Bouvet et al., 1989).

Glycerol dehydrogenase test (Bouvet et al., 1995a) For the detection of glycerol dehydrogenase, bacteria are grown overnight at 30°C on tryptocasein soy agar plates (TCS, BioRad, Marnes-la-Coquette, France) supplemented with 1% glycerol or 70 mM hydroxyacetone. Bacterial growth is collected, suspended in reaction buffer [0.1 M K_2CO_3 and 30 mM $(NH_4)_2SO_4$, adjusted to pH 9.0] to an absorbance of 0.6, and bacterial suspensions are dispensed into 96-well microtiter plates. Then, 30-μl aliquots of a reagent (NAD^+, 210 mg; glycerol, 600 ml; nitro-blue tetrazolium, 42 mg; phenazine methosulfate, 2 mg; distilled water to 10 ml) are added to the wells and incubated in the dark with shaking. The plate is examined for a purple color developed within 15 to 30 min (presence of a glycerol dehydrogenase). The color in the uninoculated control medium remains yellow. Suggested control strains are the type strain of *Serratia liquefaciens* (glycerol dehydrogenase induced by glycerol, not by hydroxyacetone) and the type strain of *Enterobacter asburiae* (dehydrogenase induced by hydroxyacetone, not by glycerol) (Bouvet et al., 1995a).

Voges–Proskauer test (Richard's modification) Clark and Lubs medium (BBL) is dispensed in large 22 × 215 mm tubes (0.5 ml per tube) and inoculated with 0.05 ml of a heavy bacterial suspension in distilled water. After incubation at 30°C for 18 h, 0.5 ml of α-naphthol solution (6% w/v alcoholic solution) and 0.5 ml of 4 M NaOH are added. The tubes are shaken, heated a few seconds in a Bunsen flame, and examined for a red color (Richard, 1972).

Tetrathionate reduction The medium of Le Minor et al. (1970a) contains: peptone (Difco), 10.0 g, NaCl, 5.0 g; $K_2S_4O_6$, 5.0 g; bromothymol blue (0.2% aqueous solution), 25 ml; and distilled water to 1 liter. Adjust the pH to 7.4, sterilize by filtration, and dispense into 12 × 120 mm tubes (4 ml per tube). The size of the tubes (for a rather limited aeration) is critical. Inoculated tubes are incubated at 30°C for 24 h and examined for a yellow color (tetrathionate reduction).

β-Xylosidase Paper disks (0.5 cm) are loaded with 0.1 ml of a 2% (w/v) aqueous solution of *p*-nitrophenyl-β-D-xylopyranoside and kept dry in a tightly-capped flask at 4°C. The test is performed exactly like the β-galactosidase test, but with *p*-nitrophenyl-β-D-xylopyranoside disks in place of ONPG disks (Brisou et al., 1972).

H-Immobilization test The motility of each isolate to be typed must be enhanced by passage through a 0.3% semisolid agar U-tube.

The following autoclaved semisolid medium is dispensed in 2.0-ml volumes into small (92 × 13 mm) screw-capped tubes: tryptic peptone, 20.0 g; D-mannitol, 2.0 g; KNO_3, 1.5 g; phenol red solution (1%), 4 ml; agar, 4.5 g. distilled water, 1000 ml; pH 7.4. The tubes of semisolid medium are melted (boiling water bath), cooled to 50°C in a water bath, supplemented with 0.05 ml of each serum dilution under sterile conditions, and allowed to gel.

Tubes with serum dilutions (and control tubes without serum) are stab-inoculated with a highly motile culture. After overnight incubation, tubes are examined for immobilization. This H-immobilization test is very specific and much easier to perform than the classical H-agglutination (Le Minor and Pigache, 1977).

Differentiation of the genus *Serratia* from other genera

Table BXII.γ.265 provides the primary characteristics that can be used to differentiate the genus *Serratia* from biochemically similar taxa.

Taxonomic Comments

A number of changes have been made since the eighth edition of the *Manual of Determinative Bacteriology*, in which it was indicated that the genus *Serratia* was composed of only one species, *S. marcescens* (the type species). Objective approaches such as numerical taxonomy and DNA relatedness applied to strains recovered from diverse habitats delineated an increasing number of species in the genus. Seven species were mentioned in the first edition of the *Bergey's Manual of Systematic Bacteriology* (Grimont and Grimont, 1984) and 10 species were mentioned in the second edition of *The Prokaryotes* (Grimont and Grimont, 1995). Ten species are currently known to belong in the genus *Serratia*.

Transfer of *Enterobacter liquefaciens* to the genus *Serratia* was first proposed by Barbe (1969) and supported by studies on bacteriocin cross-reactions between *S. marcescens* and *E. liquefaciens* (Hamon et al., 1970). Valid publication of the new combination *S. liquefaciens* followed a numerical taxonomy study (Bascomb et al., 1971).

A phenon named "biovar 2" (Bascomb et al., 1971) and "phenon B" (Grimont and Dulong de Rosnay, 1972) was thought identical to *"Bacterium rubidaeum"* Stapp 1940 and named *S. rubidaea* (Ewing et al., 1973). The same phenon was also identified as *S. marinorubra* Zobell and Upham 1944 (Grimont et al., 1977b). *S. rubidaea* and *S. marinorubra* were based on different type strains (ATCC 27593 and ATCC 27614, respectively). The *Approved Lists of Bacterial Names*, however, gives both names (*S. rubidaea* and *S. marinorubra*) with the same type strain (viz. ATCC 27614, the type strain of *S. rubidaea*). Hence, both names, which were subjective synonyms, are now objective synonyms and redundant. To avoid further confusion, the name *S. rubidaea* (Stapp) Ewing (1986a) should now be used exclusively to designate the same (*S. rubidaea–S. marinorubra*) taxon.

The ancient species *S. plymuthica* (Lehmann and Neumann 1896) Breed, Murray, and Hitchens 1948 was shown to be a valid species by numerical taxonomy (Grimont et al., 1977b) and by DNA–DNA hybridization (Grimont et al., 1978a).

Three species, *S. odorifera* (Grimont et al., 1978a), *S. ficaria* (Grimont et al., 1979b), and *S. entomophila* (Grimont et al., 1988), were defined by DNA relatedness, carbon source utilization tests, and by standard biochemical tests. DNA relatedness studies have shown that *S. marcescens*, *S. plymuthica*, *S. rubidaea*, *S. odorifera*, and *S. ficaria* are homogeneous and discrete genomospecies (Steigerwalt et al., 1976; Grimont et al., 1978a, 1979b, 1988).

S. liquefaciens sensu lato was shown to be heterogeneous (Steigerwalt et al., 1976) and later found to be composed of several genomospecies. One biovar (Clc) of *S. liquefaciens* was identified as *Erwinia proteamaculans* (Paine and Stanfield 1919) Dye 1966a and renamed *S. proteamaculans* (Grimont et al., 1978b). When DNA binding ratios were examined without studies on the thermal stability of hybridized molecules, *S. proteamaculans* was thought to be a subjective synonym of *S. liquefaciens* (Grimont et al., 1978b). Reexamination of DNA relatedness (including thermal stability studies) in *S. liquefaciens sensu lato* disclosed at least

TABLE BXII.γ.265. Differential characteristics of the genus *Serratia* and other biochemically similar taxa[a]

Characteristics	*Serratia* spp.[b]	*Serratia fonticola*	*Pantoea*	*Enterobacter cloacae*	*Pectobacterium*[c]	*Klebsiella*[d]
Carbon source utilization:[e]						
4-Aminobutyrate (M)	+	−	+	d	−	d
4-Aminovalerate (B,M)	−	−	−	−	−	d
Arginine (M)	−	−	−	−	−	+
Caprate (M)	+	−	−	−	−	−
Caproate (M)	+	−	−	−	−	−
Caprylate (M)	+	−	−	−	−	−
D-Dulcitol (B,M)	−	+	−	−	−	−
L-Fucose (B,M)	+	−	−	−	−	+
3-Phenylpropionate (B,M)	−	+	−	−	−	−
Tagatose (B,M)	−	+	−	−	−	d
Tyrosine (M)	+	-	−	−	−	d
Voges–Proskauer test	+	−	+	+	+	+
Gelatin hydrolyzed	+	−	d	d	d	d
Tributyrin hydrolyzed	+	−	−	−	−	−
Deoxyribonuclease	+	−	−	−	d	−
Gluconate dehydrogenase	+	+	+	−	−	d
Mol% G+C of DNA	52–60	49–52	53–56	53	51–54	54–57

[a]Symbols: see standard definitions.

[b]Except *S. fonticola*.

[c]According to Bouvet et al. (1989).

[d]Including *Klebsiella pneumoniae* and *K. mobilis* (*Enterobacter aerogenes*).

[e]Determined in Biotype-100 (B) or minimal agar (M).

three genomospecies: *S. liquefaciens sensu stricto, S. proteamaculans* (Grimont et al., 1982b), and a third group containing strain ATCC 14460 and named *S. grimesii* (Grimont et al., 1982a, b).

A group of strains called "*Citrobacter* lysine+" or "*Citrobacter*-like" was found to be significantly related to the genus *Serratia* in DNA–DNA hybridization studies (Crosa et al., 1974). This genomospecies has been named *Serratia fonticola* Gavini et al. 1979; however, a difficulty is that *S. fonticola* does not have the key characteristics of the genus *Serratia*. Furthermore, *Serratia* phages that are active on strains of any *Serratia* species (as defined herein) have been found to be inactive on all *S. fonticola* strains tested (unpublished data). Bacteriocins from *Serratia* are also inactive on *S. fonticola* (Hamon, personal communication). However, the 16S rRNA gene sequence of *S. fonticola* branches within the psychrotolerant *Serratia* cluster (*S. liquefaciens, S. proteamaculans, S. grimesii*, and *S. plymuthica*) and this justifies the inclusion of *S. fonticola* in the genus *Serratia* (Dauga et al., 1990).

Further Reading

Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th Ed., Elsevier, New York.

Grimont, P.A.D. and F. Grimont. 1978. The genus *Serratia*. Annu. Rev. Microbiol. *32*: 221–248.

Grimont, F. and P. Grimont. 1995. The genus *Serratia*. *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, Springer-Verlag, New York. pp. 2822–2848.

Grimont, P.A.D., F. Grimont, H.L.C. Dulong de Rosnay and P.H.A. Sneath. 1977. Taxonomy of the genus *Serratia*. J. Gen. Microbiol. *98*: 39–66.

von Graevenitz, A. and S.J. Rubin (Editors). 1980. The genus *Serratia*, CRC Press, Boca Ration, Florida.

Differentiation of the species of the genus *Serratia*

The differential characteristics of the species of *Serratia* are indicated in Table BXII.γ.266. Other characteristics of the species are listed in Table BXII.γ.267.

List of species of the genus Serratia

1. **Serratia marcescens** Bizio 1823, 288[AL]
mar.ces' cens. M.L. v. *marcesco* to fade; L. part. adj. *marcescens* fading away.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin or pyrimine can be produced.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

A biotyping system based on pigment production, tetrathionate reduction and utilization of *meso*-erythritol, trigonelline, quinate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, and DL-carnitine as sole carbon sources, has been described (Grimont and Grimont, 1978a). DL-carnitine is not in Biotype-100 strips and can be replaced by D-malate and *meso*-tartrate. Groups of biovars (called biogroups) (Table BXII.γ.268) correspond to definite, nonoverlapping sets of serovars (Table BXII.γ.269) (Grimont et al., 1979a).

Nonpigmented biogroups A3 and A4 are ubiquitous. Nonpigmented biogroups A5/8 and TCT are almost confined to hospitalized patients. Pigmented biogroups Al and A2/6 are found in the natural environment and occasionally in human patients.

The mol% G + C of the DNA is: 57.5–60 (T_m, Bd).

Type strain: ATCC 13880, CIP 103235, DSM 30121, DSM 47, JCM 1239, NCDC 813-60, NCIB 9155, NCTC 10211.

GenBank accession number (16S rRNA): AJ233431, M59160.

2. **Serratia entomophila** Grimont, Jackson, Ageron and Noonan 1988, 5[VP]
en.to.mo' phi.la. Gr. n. *entomon* insect; Gr. v. *phylein* love; L. fem. adj. *entomophila* insect loving.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Two biotypes can be delineated (Table BXII.γ.270).

Isolated from larvae of *Costelytra zealandica* (grass grub) with amber disease, and from the environment. No strain has been identified as being involved in a human, animal (other than insect), or plant disease.

The mol% G + C of the DNA is: 58 (T_m).

Type strain: A1, ATCC 43705, CIP 102919, DSM 12358.

GenBank accession number (16S rRNA): AJ233427.

3. **Serratia ficaria** Grimont, Grimont and Starr 1981d, 216[VP] (Effective publication: Grimont, Grimont and Starr 1979b, 282.)
fi.ca' ri.a. M.L. fem. adj. *ficaria* of figs.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced. Cultures give off a musty, potato-like odor.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Associated with the fig/fig-wasp biological cycle. Occasionally found on plants other than fig trees.

The mol% G + C of the DNA is: 59.6 (T_m).

Type strain: ATCC 33105, CIP 79-23, DSM 4569, ICPB 4050, JCM 1241.

GenBank accession number (16S rRNA): AJ233428, AB004745.

4. **Serratia fonticola** Gavini, Ferragut, Izard, Trinel, Leclerc, Lefebvre and Mossel 1979, 98[AL]
fon.ti' co.la. M.L. n. *fons, fontis* spring, fountain; L. suff. *-cola* dweller; M.L. n. *fonticola* spring-dweller.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced.

Does not share the key characteristics of the genus *Serratia*.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Occurs in freshwater.

The mol% G + C of the DNA is: 48.8–52.5 (T_m).

Type strain: ATCC 29844, CIP 78.64, CCUG 37824, DSM 4576, JCM 1242, LMG 7882.

GenBank accession number (16S rRNA): AJ233429.

TABLE BXII.γ.266. Characteristics differentiating the species of the genus *Serratia*[a]

Characteristics	1. *S. marcescens*	2. *S. entomophila*	3. *S. ficaria*	4. *S. fonticola*	5. *S. grimesii*	6. *S. liquefaciens*	7. *S. odorifera*	8. *S. plymuthica*	9. *S. proteamaculans*	10. *S. rubidaea*
Prodigiosin production	d	−	−	−	−	−	−	d	−	+
Potato-like odor	−	−	+	−	−	−	+	−	−	d
Indole production	−	−	−	−	−	−	+	−	−	−
Lysine decarboxylase	+	−	−	+	+	+	+	−	+	d
Ornithine decarboxylase (Møller)	+	−	−	+	+	+	d	−	+	−
Arginine decarboxylase (Møller)	−	−	−	−	+	−	−	−	−	−
Tween 80 hydrolysis	+	+	+	+	+	+	−	+	+	+
Malonate test	−	−	−	+	−	−	−	−	−	d
Carbon source utilization:										
Adonitol	+	+	+	+	−	−	+	−	d	+
L-Arabinose	−	−	+	+	+	+	+	+	+	+
D-Arabitol	−	d	+	+	−	−	−	−	−	+
L-Arabitol	+	d	+	+	−	−	+	−	−	−
Betaine	−	−	−	−	−	−	−	d	−	+
D-Cellobiose	−	+	+	d	d	+	+	+	d	+
Dulcitol	−	−	−	+	−	−	−	−	−	−
i-Erythritol	d	−	+	+	−	−	d	−	d	+
L-Fucose	+	+	+	−	+	+	+	+	+	+
Gentiobiose	−	+	+	+	d	+	d	+	+	+
Itaconate	−	+	−	−	−	−	−	−	−	−
5-Ketogluconate	+	+	+	d	+	+	d	d	+	−
Malitol	−	−	+	d	+	d	−	+	+	+
D-Melezitose	−	−	+	−	d	d	−	+	+	d
D-Melibiose	−	−	+	+	+	+	+	+	+	+
Mucate	−	−	+	d	−	−	+	d	−	+
Palatinose	−	−	+	+	+	d	−	+	+	+
3-Phenylpropionate	−	−	−	d	−	−	−	−	−	−
Quinate	d	d	+	−	−	−	−	+	−	+
D-Raffinose	−	−	+	+	+	+	d	+	+	+
L-Rhamnose	−	−	+	+	−	−	+	−	d	−
D-Saccharate	−	−	+	+	−	−	+	d	−	+
D-Sorbitol	+	−	+	+	+	+	+	d	d	−
D-Tagatose	−	−	−	+	−	−	−	−	−	−
meso-Tartrate	d	−	−	−	−	+	+	−	−	−
Trigonelline	d	−	d	−	−	−	+	−	−	+
D-Turanose	−	−	+	d	+	d	−	+	+	+
Xylitol	+	−	+	d	−	−	d	−	−	d
D-Xylose	−	d	+	d	+	+	+	+	+	+

[a]Data from Grimont and Grimont (1995) and Gavini et al. (1979). For symbols see standard definitions.

5. **Serratia grimesii** Grimont, Grimont and Irino 1983b, 438[VP] (Effective publication: Grimont, Grimont and Irino 1982a, 73.)
gri.me' sii. M.L. gen. masculine form of Grimes.

S. grimesii has been isolated from the natural environment and from human clinical specimens.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Biogroup ADC might represent a subspecies of *S. grimesii.*

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 14460, CIP 103361, DSM 30063, IFO 13537, JCM 5910.

GenBank accession number (16S rRNA): AJ233430.

6. **Serratia liquefaciens** (Grimes and Hennerty 1931) Bascomb, Lapage, Willcox and Curtis 1971, 293[AL] (*"Aerobacter liquefaciens"* Grimes and Hennerty 1931, 93.)
li.que.fa' ciens. M.L. part. adj. *liquefaciens* dissolving.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

S. liquefaciens sensu lato was composed of several biovars and some of these were found to constitute genomospecies which were subsequently given species status: biovar C1ab (including the type strain of *S. liquefaciens*) corresponded to *S. liquefaciens sensu stricto*; biovars C1c (including the type strain of *S. proteamaculans*), EB, RB, and RQ corresponded to *S. proteamaculans*; and biovars C1d and ADC corresponded to *S. grimesii* (Table BXII.γ.271) (Grimont et al., 1977b, 1982a, b).

S. liquefaciens is the most prevalent *Serratia* species in the natural environment (plants, digestive tract of rodents). Occasionally encountered as an opportunistic pathogen.

The mol% G + C of the DNA is: 53–54 (T_m, Bd).

Type strain: ATCC 27592, CIP 103238, DSM 4487, JCM 1245, LMG 7884, NCTC 12962.

GenBank accession number (16S rRNA): AJ306725, AB004752.

It should be mentioned that the strain formerly considered to be the type strain of *"Aerobacter liquefaciens"* or *"Aerobacter lipolyticus"* by Grimes (1961) was ATCC 14460 (now the type strain of *S. grimesii*). Since this strain was considered atypical compared to other strains labeled as *S. liquefaciens*, another strain (ATCC 27592) was given as type strain of *S. liquefaciens* in the Approved Lists.

TABLE BXII.γ.267. Other characteristics of the species of the genus *Serratia*[a]

Characteristics	1. *S. marcescens*	2. *S. entomophila*	3. *S. ficaria*	4. *S. fonticola*	5. *S. grimesii*	6. *S. liquefaciens*	7. *S. odorifera*	8. *S. plymuthica*	9. *S. proteamaculans*	10. *S. rubidaea*
Carbon source utilization:										
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+	+	+	+
cis-Aconitate	+	+	+	+	+	+	+	+	+	+
trans-Aconitate	+	+	+	d	−	−	+	d	d	+
D-Alanine	+	+	+	d	+	+	+	+	+	+
L-Alanine	+	+	+	d	d	+	+	+	+	+
4-Aminobutyrate	d	d	+	−	+	+	+	d	+	+
5-Aminovalerate	−	−	−	−	−	−	−	−	−	−
L-Aspartate	+	+	+	d	+	+	+	+	+	+
Benzoate	d	d	d	−	d	−	−	d	d	d
Caprate	+	d	+	−	d	+	d	−	d	d
Caprylate	d	d	+	−	d	d	d	−	d	d
Citrate	+	+	+	+	+	+	+	+	+	+
m-Coumarate	−	−	−	−	−	−	−	−	−	−
Ethanolamine	−	−	−	−	−	−	−	−	−	−
D-Fructose	+	+	+	+	+	+	+	+	+	+
Fumarate	+	+	+	d	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+
D-Galacturonate	+	+	+	+	+	+	+	+	+	+
Gentisate	d	−	−	d	−	−	−	−	d	−
D-Gluconate	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+
D-Glucuronate	+	+	+	+	+	+	+	+	d	+
L-Glutamate	+	+	+	d	+	+	+	+	+	+
Glutarate	−	−	−	−	−	−	−	−	−	−
DL-Glycerate	+	+	+	+	+	+	+	d	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+
Histamine	−	−	−	−	−	−	−	−	−	d
3-Hydroxybenzoate	d	−	−	d	−	−	−	−	−	−
4-Hydroxybenzoate	d	−	d	−	−	−	−	−	−	−
3-Hydroxybutyrate	d	−	d	−	−	−	d	−	−	−
myo-Inositol	+	+	+	+	+	+	+	+	+	+
2-Ketogluconate	+	+	+	+	+	+	+	+	+	+
2-Ketoglutarate	d	d	d	d	+	d	+	d	d	d
L-Lactate	+	+	d	d	+	+	+	d	+	+
Lactose	−	−	d	d	+	d	+	+	d	+
Lactulose	−	−	−	+	d	−	d	d	−	d
D-Lyxose	+	d	d	d	+	+	−	−	+	−
D-Malate	d	d	−	d	d	+	+	d	−	d
L-Malate	+	+	+	d	+	+	+	+	+	+
Malonate	−	−	−	d	−	−	−	−	−	d
Maltose	+	+	+	+	+	+	+	+	+	+
Maltotriose	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+
1-*O*-Methyl-α-galactoside	−	−	+	+	+	+	+	+	+	+
1-*O*-Methyl-β-galactoside	d	−	d	+	d	d	+	+	−	+
3-*O*-Methyl-D-glucose	−	−	−	d	d	d	d	d	+	−
1-*O*-Methyl-α-D-glucoside	−	−	d	d	−	d	−	d	d	+
1-*O*-Methyl-β-D-glucoside	+	+	+	+	+	+	+	+	+	+
Phenylacetate	d	d	d	d	d	+	d	d	d	+
L-Proline	+	+	+	d	d	+	+	+	+	+
Protocatechuate	d	−	d	−	d	d	−	+	d	d
Propionate	d	d	d	−	−	−	−	d	−	−
Putrescine	+	+	+	+	+	+	+	d	+	+
D-Ribose	+	+	+	+	+	+	+	+	+	+
L-Serine	+	+	+	d	+	+	+	+	+	+
L-Sorbose	−	−	−	−	−	−	−	−	−	−
Succinate	+	+	+	d	+	+	+	+	+	+
Sucrose	+	+	+	d	+	+	d	+	+	+
D-Tartrate	−	−	−	d	−	−	d	−	−	d
L-Tartrate	−	−	−	−	−	−	d	−	−	d
D-Trehalose	+	+	+	+	+	+	+	+	+	+
Tricarballylate	−	−	−	d	−	−	−	−	−	d
Tryptamine	−	−	−	−	−	−	−	−	−	−
L-Tyrosine	d	d	+	−	d	d	+	d	d	+

(*continued*)

TABLE BXII.γ.267. *(cont.)*

Characteristics	1. *S. marcescens*	2. *S. entomophila*	3. *S. ficaria*	4. *S. fonticola*	5. *S. grimesii*	6. *S. liquefaciens*	7. *S. odorifera*	8. *S. plymuthica*	9. *S. proteamaculans*	10. *S. rubidaea*
Oxidation of:										
Glucose to gluconate (without cofactor)	+	+	+	−	−	−	+	d	−	+
2-Ketogluconate to 2,5-diketogluconate	+	−	−	−	+	+	−	−	−	−
Glycerol dehydrogenase induced by glycerol, not hydroxyacetone	+	−	−	+	+	+	−	−	+	−
Acid produced from:										
Adonitol	d	+	+	+	−	−	v	−	−	+
L-Arabinose	−	−	+	+	+	+	+	+	+	+
D-Melibiose	−	−	+	+	+	+	+	+	+	+
myo-Inositol	d	−	+	+	+	+	+	d	+	d
D-Raffinose	−	−	+	+	+	+	d	+	+	+
L-Rhamnose	−	−	+	v	−	−	+	−	−	−
D-Sorbitol	+	−	+	+	+	+	+	d	+	−
Sucrose	+	+	+	−	+	+	d	+	+	+
D-Xylose	−	d	+	d	+	+	+	+	+	+
Growth at 5°C	−	+	+	+	+	+	+	+	+	−
Growth at 37°C	+	+	+	+	+	+	+	d	+	+
Growth at 40°C	+	+	−	+	−	−	+	−	−	d
Growth in NaCl:										
7% (w/v)	+	+	+	+	d	d	+	d	d	+
8.5% (w/v)	d	−	−	−		−	+	−	−	+
10% (w/v)	−	−	−	−	−	−	−	−	−	d
Tetrathionate reduced	d	−	−	+	+	+	−	−	+	−
Gas from glucose	−	−	−	d	+	+	−	d	+	−
β-Xylosidase	−	−	d	+	−	−	+	d	−	+
Tween 40 hydrolysis	+	+	+	+	+	+	+	+	+	+
Tween 60 hydrolysis	+	+	+	+	+	+	d	+	+	+
Chitin hydrolysis	+	d	+	−	d	d	−	+	d	−
Methyl red test	−	−	−	+	d	d	+	d	d	−
L-Histidine deaminase	−	−	−	−	−	−	−	−	−	−
Tryptophan deaminase	−	−	−	−	−	−	−	−	−	−
β-glucuronidase	−	−	−	−	−	−	−	−	−	−
Esculin hydrolysis	+	+	+	+	+	+	+	+	d	+

[a]Data from Grimont and Grimont (1995) and Gavini et al. (1979). For symbols see standard definitions.

TABLE BXII.γ.268. Identification of *S. marcescens* biogroups and biovars[a]

	Biogroups																	
	A1		A2		A6	A3				A4		A5	A8					
Characteristics	a	b	a	b	a	a	b	c	d	a	b		a	b	c	TCT	TC	TT
Prodigiosin production	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth on:[b]																		
meso-Erythritol	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Benzoate	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Quinate and/or 4-hydroxybenzoate	−	−	−	−	+	−	−	−	−	+	−	+	+	+	+	−	−	−
3-Hydroxybenzoate	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−
Trigonelline	−	−	−	−	−	−	+	−	+	−	−	+	+	+	+	+	−	+
D-Malate/*m*-tartrate	+	−	−	+	d	−	−	d	−	+	+	+	d	−	−	+	+	−
Gentisate	−	−	+	+	+	+	+	d	−	+	+	−	d	+	d	−	−	−
Tetrathionate reduction	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+

[a]For symbols see standard definitions.

[b]Carbon source utilization test.

7. **Serratia odorifera** Grimont, Grimont, Richard, Davis, Steigerwalt and Brenner 1978a, 461[AL]

o.do.ri.fe' ra. M.L. fem. adj. *odorifera* bringing odors, fragrant.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced. Cultures give off a musty, potato-like odor.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267. Two biovars can be recognized (Table BXII.γ.272).

The capsular antigen reacts with *Klebsiella* antisera K4 or K68.

Rare opportunistic pathogen. Occasionally isolated from plants or food.

The mol% G + C of the DNA is: 54.6 (T_m).

TABLE BXII.γ.269. Correspondence between serovars and biogroups in *Serratia marcescens*

Biogroup[a]	O:H serovars[b]
A1	5:2; 5:3; 5:13; 5:23; 10:6; 10:13; 28:2
A2/6	5:23; 6,14:2; 6,14:3; 6,14:8; 6,14:9; 6,14:10; 6,14:13; 8:3; 13:5
A3	3:5; 3:11; 4:5; 4:18; 5:6; 5:15; 6,14:5; 6,14:6; *6,14:16*; 6,14:20; *9:9*; *9:11*; *9:15*; 9:17; 12:5; 12:9; *12:10*; 12:11; *12:15*; *12:16*; 12A:17; *12:18*; 12:20; *12:26*; 13:11; *13:17*; 15:3; 15:5; 15:8; 15:9; 17:4; 18:21; *18:26*; *22:11*; *23:19*, 26:20
A4	1:1; 1:4; 2:1; 2:8; 3:1; 4:1; 4:4; 5:1; 5:6; 5:8; 5:24; 9:1; 13:1; 13:11; 13:13
A5/8	*2:4*; 3:12; 3,21:12; 4:12; 5:4; 6,14:4; 6,14:12; *8:4*; 8:12; 15:12; 21:12, 25:12
TCT	*1:7*; 2:7; *4:7*; 5:7; 5:19; *7:7*; 7:23; 10:9; 11:4; 13:7; 13:12; 16:19; *18:9*; 18:16; *18:19*; 19:14; *19:19*; 24:6; 27:−
TC	10:8; 20:12

[a]Biogroup A1 is composed of biotypes A1a and A1b; A2/6 of A2a, A2b, A6; A3 of A3a, A3b, A3c, A3d; A4 of A4a, A4b; A5/8 of A5, A8a, A8b, and A8.

[b]Serovars for which exceptions to the correspondence occur are in italics.

TABLE BXII.γ.270. Identification of *Serratia entomophila* biotypes[a]

Characteristics	Biotype 1b	Biotype 2
Growth on:[b]		
D-Arabitol	+	−
L-Arabitol	−	+
D-Malate	−	d
D-Sorbitol	−	d
Quinate	+	d
D-Xylose	−	+

[a]For symbols see standard definitions.

[b]The type strain corresponds to biotype 1.

Type strain: ATCC 33077, CIP 79-1, DSM 4582, ICPB 3995, NCTC 11214.

GenBank accession number (16S rRNA): AJ233432.

8. **Serratia plymuthica** (Lehmann and Neumann 1896) Breed, Murray and Hitchens 1948, 481^{AL} (*Bacterium plymuthicum* (sic) Lehmann and Neumann 1896, 264.)
ply.mu'thi.ca. M.L. adj. *plymuthica* pertaining to Plymouth, UK.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is produced by most strains.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Most *S. plymuthica* strains studied were isolated from freshwater. Very rarely found in human sputum. No human infection reported.

The mol% G + C of the DNA is: 53.5–56.5 (T_m).

Type strain: ATCC 183, CIP 103239, DSM 4540, JCM 1244.

GenBank accession number (16S rRNA): AJ233433.

S. plymuthica is cited on the Approved Lists of Bacterial Names (Skerman et al., 1980) as *Serratia plymuthica* (Dyar 1895) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 88. This is incorrect, for reasons discussed by Grimont et al. (1977b).

9. **Serratia proteamaculans** (Paine and Stansfield 1919) Grimont, Grimont and Starr 1978b, 503^{AL} (*Pseudomonas proteamaculans* Paine and Stansfield 1919, 38.)
pro.te.a.ma.cu'lans. M.L. n. *Protea* a plant generic name; L. v. *maculo* to spot; M.L. part. adj. *proteamaculans* spotting *Protea.*

S. proteamaculans and *S. liquefaciens* were thought to be synonymous based on DNA relatedness (Grimont et al., 1978b). However, subsequent observation of significant thermal instability of reassociated DNA fragments supported the separation of both species (Grimont et al., 1982b).

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

S. proteamaculans is found in the natural environment (plants, wild rodents, insects, and water) but exceptionally from human clinical specimens).

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 19323, CIP 103236, DSM 4543, NCPPB 245.

According to J-P. Euzeby (http://www.bacterio.cict.fr/corrections2.html), the spelling should have been *proteimaculans.* However, since the name appeared in the Approved Lists, no correction is allowed.

a. **Serratia proteamaculans** *subsp.* **proteamaculans** (Paine and Stansfield 1919) Grimont, Grimont and Starr 1978b, 503^{AL} (*Pseudomonas proteamaculans* Paine and Stansfield 1919, 38.)

The subspecies includes biovars C1c, EB, and RB. The type strain belongs to biotype C1c. Differential characteristics are given in Table BXII.γ.271.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 19323, CIP 103236, DSM 4543, NCPPB 245

GenBank accession number (16S rRNA): AJ233434.

b. **Serratia proteamaculans** *subsp.* **quinovora** Grimont, Grimont and Irino 1983b, 438^{VP} (Effective publication: Grimont, Grimont and Irino 1982a, 71.)
qui.no'vo.ra. M.L. *quinate,* from Spanish *quina* quinine; and L. v. *voro* to devour; M.L. fem. adj. *quinovora* quinate devouring.

This subspecies corresponds to biotype RQ. Quinate utilization was observed for all strains of this subspecies in a minimal agar when an unwashed inoculum was used. In Biotype-100 strips, no growth is observed on quinate when Biotype Medium 1 is used, some strains grow from quinate when Biotype Medium 2 (containing more growth factors) is used.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 33765, CIP 81-95, DSM 4597.

GenBank accession number (16S rRNA): AJ233435.

10. **Serratia rubidaea** (Stapp 1940) Ewing, Davis, Fife and Lessel 1973, 224^{AL} (*"Bacterium rubidaeum"* Stapp 1940, 259; *Serratia marinorubra* Zobell and Upham 1944, 255.)
ru.bi'dae.a. L. *Rubus idaeus* raspberry, contracted and made to agree in gender with *Serratia.*

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is produced by most strains.

Physiological and nutritional characteristics are presented in Tables BXII.γ.266 and BXII.γ.267.

Three biotypes (B1, B2, B3) correspond to subspecies "*S. rubidaea* subsp. *burdigalensis*", "*S. rubidaea* subsp. *rubidaea*", and "*S. rubidaea* subsp. *colindalensis*" (Grimont et al., manuscript in preparation) (Table BXII.γ.273).

S. rubidaea strains are rarely isolated, either in the natural environment or in human patients. May be found in ripe coconuts (Grimont et al., 1981c).

The mol% G + C of the DNA is: 53.5–58.5 (T_m).

Type strain: Ewing 2199-72, ATCC 27593, CIP 103234, DSM 4480, JCM 1240.

GenBank accession number (16S rRNA): AB004751, AJ233436.

TABLE BXII.γ.271. Identification of species in the *Serratia liquefaciens* complex[a]

Characteristics	*S. liquefaciens* C1ab	*S. proteamaculans* subsp. *proteamaculans* C1c[b]	EB	RB	*S. proteamaculans* subsp. *quinovora* RQ	*S. grimesii* C1d[c]	ADC
Carbon source utilization test:[d]							
trans-Aconitate	−	+	+	d	+	−	+
Benzoate	−	−	d	d	−	+	−
m-Erythritol	−	−	+	−	−	−	−
Gentisate	−	−	−	+	−	−	−
D-Malate	+	−	−	−	d	v	v
L-Rhamnose	−	−	−	+	d	−	−
m-Tartrate	+	−	−	−	d	−	−
Arginine decarboxylase	−	−	−	−	−	+	+
Esculin hydrolysed	+	+	+	+	−	+	+

[a]For symbols see standard definitions.

[b]The type strain of *S. proteamaculans* (ATCC 19323) corresponds to biotype C1c.

[c]The type strain of *S. grimesii* (ATCC 14460) corresponds to biotype C1d.

[d]Biotype-100 strips.

TABLE BXII.γ.272. Identification of *Serratia odorifera* biotypes[a]

Characteristics	Biotype 1[b]	2
Growth on:		
m-Erythritol	−	+
L-Fucose	d	+
D-Raffinose	+	−
Sucrose	+	−
D-Tartrate	+	−
Ornithine decarboxylase	+	−
Acid from sucrose	+	−
Acid from raffinose	+	−[c]

[a]For symbols see standard definitions.

[b]The type strain corresponds to biotype 1.

[c]Some strains positive in 3–7 days.

TABLE BXII.γ.273. Identification of *Serratia rubidaea* biotypes[a]

Characteristics	Biotype[b] B1	B2	B3
Growth on:			
Histamine	d	−	d
D-Melezitose	−	+	+
D-Tartrate	+	−	d
Tricarballylate	−	d	−
Voges–Proskauer (O'Meara)	+	−	d
Lysine decarboxylase	+	+	−
Malonate (Leifson)	+	+	−

[a]For symbols see standard definitions.

[b]The biotypes were shown to correspond to subspecies designated as "*S. rubidaea* subsp. *burdigalensis*" (B1), "*S. rubidaea* subsp. *rubidaea*" (B2), and "*S. rubidaea* subsp. *colindalensis*" (B3).

Genus XXXV. **Shigella** *Castellani and Chalmers 1919, 936*[AL]

NANCY A. STROCKBINE AND ANTHONY T. MAURELLI

Shi.gel' la. M.L. dim. *-ella* ending; M.L. fem. n. *Shigella* named after K. Shiga, the Japanese bacteriologist who first discovered the dysentery bacillus.

Straight rods, 1–3 × 0.7–1.0 μm, that conform to the general definition of the family *Enterobacteriaceae* and contain the enterobacterial common antigen. Gram negative. Nonmotile. Nonpigmented. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Catalase positive (with exceptions in *Shigella dysenteriae*). Oxidase negative. Chemoorganotrophic. **Ferment sugars without gas production** (a few exceptions produce gas). **Salicin, adonitol, and *myo*-inositol are not fermented**. Strains of *Shigella sonnei* ferment lactose and sucrose upon extended incubation; however, other species do not utilize these substances in conventional medium. **Do not utilize citrate, malonate, or sodium acetate** (with exceptions in *Shigella flexneri* for sodium acetate) as a sole carbon source. **Do not grow in KCN or produce H_2S. Do not decarboxylate lysine**. Reduce nitrates to nitrites. Intestinal pathogens of humans and other primates, causing bacillary dysentery. Based on 16S rDNA sequencing, shigellae belong in the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 49–53 (Laskin and Lechevalier, 1981).

Type species: **Shigella dysenteriae** (Shiga 1898) Castellani and Chalmers 1919, 935, Epit. Spec. Cons. Opinion 11 of the Jud. Comm. 1954a, 149 (*Bacillus dysenteriae* Shiga 1898, 817.)

Further descriptive information

Phylogenetic and systematic treatment Scientific evidence accumulated to date strongly supports the view that *Shigella* species are biotypes/pathotypes or clones of *Escherichia coli*. As early as 1957, Luria and Burrous (1957) showed that *E. coli* and the majority of *Shigella* species are in the same fertility system and suggested that genetic recombination between these organisms could play a role in the evolution of some *Shigella* serotypes and, more generally, in the evolution of natural populations of these bacteria. In the early 1970s, Brenner et al. (1972a, 1973a) published findings from DNA–DNA reassociation studies that provided the first direct insights into the evolutionary relationships between shigellae and *E. coli*. These authors found that, with the exception of *S. boydii* 13 (see discussion under Taxonomic comments), *Shigella* species were as related to *E. coli* (>75% nucleotide similarity) as they were to each other.

Given the variability that occurs within the defined *E. coli* species, the question then is whether the four species of *Shigella* represent the evolution of new species from a common ancestor or whether these organisms are clones of *E. coli*. Findings from multilocus enzyme electrophoresis (MLEE) analyses strongly support the conclusion that shigellae are more appropriately regarded as pathotypes or clones of *E. coli*. Principal component analysis of 302 electrophoretic types (ETs) from more than 1600 human and animal isolates of *E. coli* and 123 strains of the four species of *Shigella* showed that the *Shigella* strains clustered within two of the three overlapping groups of *E. coli* strains detected, rather than forming a group apart from those comprised by *E. coli*, as would be expected of a distinct genus (Ochman et al., 1983). Furthermore, with the exception of *S. boydii* 13, strains representing all four species of *Shigella* did not cluster in groups corresponding to each species. A dendrogram of the 23 ETs from *Shigella* constructed by average linkage clustering revealed two major clusters each containing strains of three or more species; one group consisted of ETs representing *S. flexneri*, *S. dysenteriae*, and *S. boydii* strains and the other consisted of ETs representing strains from all four species, with *S. sonnei* being most distantly related (Ochman et al., 1983).

MLEE findings from the study by Pupo et al. (1997) also showed that strains from the four species of *Shigella* clustered in two separate groups, with one group containing strains from the majority of *Shigella* species and the other group containing only *S. flexneri* strains. With the exception of one *S. flexneri* serotype, the *Shigella* species appeared as clones within the *E. coli* species. Nucleotide sequence analysis of the housekeeping gene maltose dehydrogenase (*mdh*) (Pupo et al., 1997) and analysis of restriction fragment length polymorphisms of rDNA (ribotyping) (Rolland et al., 1998) also yielded largely similar clustering of shigellae, but showed somewhat different distributions of the *Shigella* spp. among the *E. coli* phylogenetic groups.

A careful analysis of 16S ribosomal DNA sequences by Cilia et al. (1996) also placed shigellae and *E. coli* in the same phylogenetic group, supporting the concept that *Shigella* species and *E. coli* are five nomenspecies that represent a single genomospecies. In fact, the variation of the 16S ribosomal RNA genes in the seven *rrn* operons of a single strain of *E. coli* shared a clade with *S. dysenteriae*, *S. flexneri*, and *S. sonnei* sequences. There were informative base alleles that were shared by both *E. coli* and *Shigella* species.

Recent findings reported by Pupo et al. (2000) from the examination of nucleotide sequence similarity at eight housekeeping genes in four regions around the chromosome of *Shigella* and selected nonpathogenic *E. coli* strains (ECOR set) revealed three clusters of closely related strains, each including exclusively *Shigella* strains but from more than one species. *S. sonnei* and three *S. dysenteriae* strains from *S. dysenteriae* serotypes 1, 8, and 10 fell outside the three main clusters but were well within the population structure of *E. coli*. The presence of these seven different groups (clusters 1–3, *S. sonnei*, *S. dysenteriae* serotypes 1, 8, and 10) within *E. coli* suggests that the *Shigella* phenotype has arisen as many as seven times from ancestors that were *E. coli*. Pupo et al. (2000) propose that the many independent origins of the *Shigella* phenotype are an example of convergent evolution. Phenotypes that may have arisen by convergent evolution include the loss of catabolic pathways involving lactose fermentation, mucate utilization, lysine decarboxylation, the loss of motility, and the acquisition of the invasion plasmid (Pupo et al., 2000).

It has been suggested that *E. coli* arose from a common ancestor with *Salmonella* and diverged largely as the result of the acquisition of multiple genomic fragments (Lawrence and Ochman, 1998). It appears that *Shigella* differs from *E. coli* largely by the loss of genomic fragments. Maurelli et al. (1998) showed that shigellae lack a large fragment of the genome relative to *E. coli* K-12. It is interesting to note that the missing fragment included the *cadA* gene discussed in more detail below. The same pattern has been noted with another genomic fragment that derives from a cryptic prophage in *E. coli* K-12 and includes the *ompT* gene (Nakata et al., 1993). This gene encodes a surface protease that when introduced into *Shigella* inhibited the ability of the organism to spread among host cells. Another fascinating deletion occurs in *S. sonnei*. A large portion of the O-antigen gene cluster has been excised, leaving remnants of genes normally found at the ends of the cluster fused together (Lai et al., 1998). The clone carries a plasmid that has O-antigen genes that apparently arose in *Plesiomonas shigelloides* (Houng and Venkatesan, 1998; Chida et al., 2000). It has been postulated that the acquisition of the new O-antigen was a prerequisite for *S. sonnei* emerging in its modern niche and that this might have occurred about 10,000 years ago (Lai et al., 1998).

In contrast to the concept of species being defined as a group having a monophyletic origin, a species may be defined as a group of organisms sharing genetic drift (Hey, unpublished studies). By this latter definition, *Shigella* species might be classified independently of *E. coli*. Factors that affect the sharing of genetic drift among bacteria are natural selection and recombinant exchange of DNA. Shigellae are considered to be host-adapted to primates and thus have a narrower niche than *E. coli*. It is difficult to conclusively define species of bacteria based on recombination-exchange partners. Islands of DNA that clearly came from other species can frequently be found in bacterial genomes. However, it does appear that the frequency of recombination is proportional to the closeness of the species. Matsutani and Ohtsubo (1993) surveyed the distribution of five insertion elements (IS1, IS600, IS629, IS630, and IS640) originally found in *S. sonnei*. In general, the elements were found in high copy numbers in *S. sonnei*, *S. dysenteriae*, *S. flexneri*, and *S. boydii* (often more than 20 copies per genome), in moderate numbers in *E. coli*, and occa-

sionally, albeit rarely, in other *Enterobacteriaceae*. Thus, it may be that *Shigella* species are in the process of drifting apart from *E. coli*. The alternative hypothesis is that the insertion elements are more readily duplicated within clones that they exist in rather than across clones. However, the conclusion is the same since it would mean that the elements are more ancient in shigellae and this is an example of genetic drift that is not shared with *E. coli*.

If history were to be rewritten with the privilege of having our current understanding of the pathogenesis and diversity extent within *E. coli*, a very different nomenclature for *Shigella* would undoubtedly be adopted. Although phylogenetically it would be better to treat shigellae as pathotypes of *E. coli*, the members of the genus *Shigella* continue to be divided for historical and medical purposes into four species or subgroups: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). The useful information communicated about the disease caused by these organisms (shigellosis) through the genus epithet *Shigella* has preserved and will likely continue to preserve the current nomenclature until such time when there is a greater need to communicate the natural relationships that exist between these strains.

Biochemical characteristics of the genus are listed in Table BXII.γ.274. The biochemical reactions that are useful for differentiating the four species of *Shigella* are listed in Table BXII.γ.275. The ability to ferment D-mannitol is a particularly important differential biochemical trait for classifying the shigellae. Although exceptional strains occur, members of subgroup A are unable to ferment D-mannitol, while members of the other subgroups (B, C, and D) are able to ferment this sugar. Members of the genus *Shigella* have distinctive antigenic structures by which they are divided into a variety of serotypes and subserotypes. It is the combination of biochemical traits, particularly the D-mannitol phenotype, and antigenic properties that have guided the classification of members of this genus. The current schema for classifying *Shigella*, which recognizes 49 serotypes and subserotypes, is shown in Table BXII.γ.276.

Cell morphology Shigellae are nonmotile, Gram-negative rods with rounded ends. They are typically 1–3 μm in length and 0.7–1 μm in diameter. Fig. BXII.γ.209 shows negatively stained preparations of *S. dysenteriae* 1 and *S. flexneri* 2a. The bacterial cells in this figure are nonflagellated, which is typical of the members belonging to this genus. Under certain culture conditions, however, some cells in a culture have been observed to express flagella. See the section below on fine structure for a discussion of exceptions to the presence of flagella.

Cell wall composition The cell walls of shigellae resemble those of other Gram-negative bacteria in structure, composition, and endotoxic activity. When serologically characterizing living (unheated) isolates that are biochemically consistent with *Shigella*, it is important to be aware that living bacterial cells of certain strains of *Shigella* can produce a substance that will block their agglutination with O antisera. The masking effects of this substance, originally referred to as a K antigen of the B variety or B antigen, can be inactivated by heat. Treatment of nonagglutinable cultures at 100°C for 1 h restores their ability to be agglutinated by O antisera. Blocking substances also occur in certain *E. coli* strains and were shown by Ørskov et al. (1977) to be chemically and antigenically indistinguishable from the O antigens present in the cell-wall lipopolysaccharides of these bacteria. Blocking substances (B antigens) are no longer recognized as distinct antigens; however, their presence in unheated cells remains important from a practical standpoint.

TABLE BXII.γ.274. Characteristics of the genus *Shigella*[a]

Test or Substrate	Result
H_2S (triple sugar iron agar)	−
Urea hydrolysis	−
Indole production	d[b]
Methyl red	+
Voges–Proskauer	−
Citrate (Simmons')	−
Citrate (Christenson's)	−
Growth in KCN	−
Motility at 37°C	−
Gelatin hydrolysis at 22°C	−
Lysine decarboxylase	−
Arginine dihydrolase	d
Ornithine decarboxylase	d[c]
Phenylalanine deaminase	−
D-Glucose:	
Acid	+
Gas	−[d]
Acid from:	
Adonitol	−
α-Methyl-D-glucoside	−
L-Arabinose	d
Cellobiose	−
Dulcitol	d
Erythritol	−
Glycerol	d
myo-Inositol	−
Lactose	d[e]
Maltose	d
D-Mannitol	d[f]
Raffinose	d
L-Rhamnose	d
Salicin	−
D-Sorbitol	d
Sucrose	d[g]
Trehalose	d
D-Xylose	d
Malonate (sodium) utilization	−
Mucate (sodium) utilization	−
Acetate (sodium) utilization	−
Esculin hydrolysis	−
β-Galactosidase (ONPG)[h]	d[i]
Nitrate to nitrite	+
Oxidase	−

[a]For symbols see standard definitions. Adapted from findings reported by Ewing (1971) based on the examination of 5166 cultures representative of all four species. Unless specified otherwise, results are for 48 h incubation at 36° ± 1°C.

[b]Some strains of some serotypes of *S. dysenteriae*, *S. flexneri*, and *S. boydii* produce indole while strains of other serotypes are always negative. *S. sonnei* is always negative.

[c]Strains of *S. boydii* 13 and *S. sonnei* are positive.

[d]Some biotypes of *S. flexneri* 6 are positive; positive strains of *S. boydii* 13 and 14 have been described.

[e]Strains of *S. sonnei* are usually positive after several days of incubation; positive strains of *S. flexneri* 2a, *S. boydii* 9, and *S. boydii* 15 have been described. Some strains of *S. dysenteriae* 1 ferment lactose slowly; however, all are positive for β-galactosidase.

[f]Strains of *S. dysenteriae* are negative; negative biotypes of *S. flexneri* 4a ("*S. rabaulensis*", "*S. rio*") and *S. flexneri* 6 (Newcastle biotype) occur; negative biotypes of *S. sonnei* occur rarely.

[g]Strains of *S. sonnei* are usually positive after several days of incubation.

[h]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[i]Strains of *S. dysenteriae* 1 and *S. sonnei* are positive; some positive strains of *S. flexneri* 2a and *S. boydii* 9 have been described.

Fine structure

Flagella By standard methods (radial spread from the site of inoculation in medium containing 0.3–0.4% agar), shigellae are nonmotile. Over the past decade, various investigators have shown that *Shigella* strains have the genes involved in producing flagella and, under certain conditions, can express these genes.

TABLE BXII.γ.275. Distinguishing biochemical reactions of the four species of *Shigella*[a]

Test	*S. dysenteriae*	*S. boydii*	*S. flexneri*	*S. sonnei*
Indole production	d	d	d	−
Arginine dihydrolase	−	d	−	−
Ornithine decarboxylase	−	−	−	+
Acid production from:				
Lactose	−	−	−	(+)[b]
Sucrose	−	−	−	(+)
D-Mannitol	−	+	+	+
Dulcitol	d	d	−	−
D-Sorbitol	d	d	d	−
Raffinose	−	−	d	(+)
D-Xylose	−	d	−	−
Melibiose	−	d	d	−
β-Galactosidase (ONPG)[c]	d	d	−	+

[a]For symbols see standard definitions. Results are for 48 h incubation at 36° ± 1°C under conditions described by Ewing (1971).

[b](+) = 75% or more of strains gave a positive reaction after 3 or more days.

[c]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

Direct evidence for the existence of flagellum-related sequences in *Shigella* was reported by Tominaga et al. (1994), who identified and characterized cryptic genes for flagellin (*fliC*) in *S. flexneri* and *S. sonnei*. The cloned *fliC* gene from *S. flexneri* produced normal-type flagella when introduced into a strain of *E. coli* deleted for *fliC*, while the cloned *fliC* gene from *S. sonnei* produced curly-type flagella in this host strain. Findings from comparisons of nucleotide sequences of the *fliC* genes from *S. flexneri* and *S. sonnei* with those from *E. coli* and *Salmonella* serotype Typhimurium revealed various regions of high similarity between these bacteria. Similarities at and near the 5′ and 3′ constant regions of the *S. flexneri* and *E. coli fliC* genes suggested the *fliC* gene from *S. flexneri* is an *E. coli* type gene, while similarity observed between the *fliC* gene of *S. sonnei* at the operator and 3′ constant region of *Salmonella* serotype Typhimurium and the downstream sequence of the *E. coli fliC* gene suggest the *S. sonnei* gene has undergone horizontal transfer and recombination (Tominaga et al., 1994). Coimbra et al. (2001) recently examined restriction fragment polymorphisms in the *fliC* genes of representative strains from each of the serotypes within the four species of *Shigella*. Seventeen F types, defined by *fliC* patterns sharing most of their bands, were observed among the 120 strains tested. Although O antigenic relationships between certain *E. coli* strains and shigellae are well documented, none of the F types observed among the tested *Shigella* strains matched the 62 F types previously described for *E. coli* (Machado et al., 2000). This method is a promising tool for the confirmation of atypical isolates as shigellae.

Prompted by reports that shigellae possessed the genes for flagella, Girón (1995) reexamined them for flagella by electron microscopy. He observed that strains of all four *Shigella* species, when cultivated under certain conditions, produced flagella. Motility was observed only by using low-concentration motility agar (0.175–0.2% agar), and genetic and environmental factors involved in the regulation of flagella are not yet identified. Expression of flagellated cells within a culture ranged from 1:300 to 1:1000, with flagellated cells typically producing one polar flagellum or occasionally 2–3 flagella in a semipolar topology. When expressed, the flagellum of *Shigella* is approximately 10 μm long and 12–14 nm in diameter and typically emanates from one pole of the bacterium. Putative flagellins of 33–38 kDa, which share immunologic similarities with *E. coli*, *Salmonella* spp., and *Proteus mirabilis* flagellins, have also been identified (Girón, 1995).

It should be emphasized, however, that shigellae are nonmotile under standard assay conditions. Al Mamun et al. (1997) showed that members of the four *Shigella* species became nonmotile by undergoing various kinds of stable mutations in different flagellar genes. Findings from these investigators suggest that the loss of flagella in *S. boydii* is attributable to a defect or deletion in the *fliF* operon, while the primary cause for nonmotility in *S. sonnei* is a deletion in the *fliD* gene. In *S. dysenteriae* and *S. flexneri*, IS1 insertions in the *flhD* master operon may be the primary cause of motility loss (Al Mamun et al., 1996).

Fimbrial adhesins In initial studies, *S. flexneri* was the only species of *Shigella* that was found to produce fimbriae (Duguid and Gillies, 1957). The fimbriae of *S. flexneri* were similar to those produced by *E. coli*; their width was uniformly about 0.01 μm and their length was between 0.3 and 2.0 μm. In these early studies, *S. flexneri* strains were found to readily undergo a reversible mutation between a fimbriate, hemagglutinating phase and a nonfimbriate, nonhemagglutinating phase. Findings by Snellings et al. (1997) confirmed the presence of fimbriae (type 1) on strains of *S. flexneri* and demonstrated their presence on recent clinical isolates of *S. boydii* and *S. dysenteriae*. Analysis of sequences upstream of the type 1 fimbriae subunit gene, *fimA*, showed that random phase variation between fimbriated and afimbriated states in *Shigella* was accompanied by the genomic rearrangement associated with phase variation in *E. coli* (Snellings et al., 1997). *Shigella* appears to switch between phases at a much lower frequency than *E. coli*, and this in combination with inappropriate growth conditions could partially explain the difficulty researchers have had demonstrating fimbriae in *Shigella*. The role fimbriae play in the pathogenesis of shigellae is not known. Some investigators have speculated that these structures may facilitate the formation of pellicles at the water–air interface in aquatic environments and thereby promote the survival of bacteria between outbreaks of disease (Snellings et al., 1997). Studies have also demonstrated the presence of type 3 and type 4 fimbrial adhesions and of afimbrial adhesions in *Shigella* strains (Qadri et al., 1989; Utsunomiya et al., 1992).

Another surface structure of note with respect to *Shigella* is the thin aggregative fimbria called curli. Curli have been demonstrated in a wide variety of *E. coli* and *Salmonella* spp. and are believed to mediate formation of biofilms or bacterial attachment to host intestinal cells. Rather than their presence, it is their complete absence from all four species of *Shigella*, as well as from enteroinvasive *E. coli* strains, that is notable here (Sakellaris et al., 2000). Shigellae are unable to produce curli because of insertions or deletions in the curli locus (*csg*); enteroinvasive *E. coli* strains are similarly affected or have another uncharacterized lesion(s) resulting in the lack of curli expression (Sakellaris et al., 2000). Since expression of curli occurs in a variety of *E. coli* strains, the widespread loss of curli from *Shigella* and enteroinvasive *E. coli* strains, which have a very similar mechanism of pathogenesis to *Shigella*, may represent a pathoadaptive mutation (Sakellaris et al., 2000). It is possible that during the divergence of *Shigella* from *E. coli*, the expression of curli in the new virulence niche was a selective disadvantage for *Shigella*.

Colonial or cultural characteristics On agar medium, colonies of *Shigella* strains can appear smooth and glistening or rough and dry. The degree of smoothness displayed depends in large part on the extent of polymerization of the O-antigen on the

TABLE BXII.γ.276. Serologic schema, antigenic formulae, and earlier designations of *Shigella* species

Subgroup and Species	Serotype/Subserotype	Antigenic Formula[a]	Main earlier designations or synonyms
Subgroup A			
S. dysenteriae	1		*"Bacterium shigae"*, *"S. shigae"* (Shiga, 1898)
	2		*"S. ambigua"*, *"S. schmitzii"* (Schmitz, 1917)
	3		*"S. largei"* Q771 (Large and Sankaran, 1934), *S. arabinotarda* A
	4		*"S. largei"* Q1167 (Large and Sankaran, 1934), *S. arabinotarda* B
	5		*"S. largei"* Q1030 (Large and Sankaran, 1934)
	6		*"S. largei"* Q454 (Large and Sankaran, 1934)
	7		*"S. largei"* Q902 (Large and Sankaran, 1934)
	8		Serotype 599-52 (Ewing et al., 1952b)
	9		Serotype 58 (Cox and Wallace, 1948)
	10		Serotype 2050-52 (Ewing, 1953)
	11		Serotype 3873-50 (Ewing and Hucks, 1952)
	12		Serotype 3341-55 (Ewing et al., 1958)
	13		Serotype I9809-73 (Shmilovitz et al., 1985)
	14		Serotype E22383 (Gross et al., 1989)
	15		Serotype E23507 (Gross et al., 1989)
Subgroup B			
S. flexneri	1a	I:4	V (Andrewes and Inman, 1919)
	1b	I:(4),6	VZ (Andrewes and Inman, 1919)
	2a	II:3,4	W (Andrewes and Inman, 1919)
	2b	II:7,8	WX (Andrewes and Inman, 1919)
	3a	III:(3,4), 6,7,8	Z (Andrewes and Inman, 1919)
	3b	III:(3,4),6	
	4a	IV:(3,4)	103 (Boyd, 1931)
	4b	IV:6	103Z
	4c	IV:7,8	(Pryamukhina and Khomenko, 1988)
	5a	V:(3,4)	P119 and P119X (Boyd, 1932a, b)
	5b	V:7,8	(Petrovskaya and Khomenko, 1979)
	6	VI:4	Boyd 88 (Boyd, 1931) Manchester bacillus, Newcastle bacillus, *"S. newcastle"*
	X	−:7,8,	X (Andrewes and Inman, 1919)
	Y	−:3,4	Y (Andrewes and Inman, 1919)
Subgroup C			
S. boydii	1		170 (Boyd, 1932a, b; Ewing, 1949)
	2		P288 (Boyd, 1932a, b; Ewing, 1949)
	3		D.1 (Boyd, 1932a, b; Ewing, 1949)
	4		P274 (Boyd, 1932a, b; Ewing, 1949)
	5		P143 (Boyd, 1938; Ewing, 1949)
	6		D19 (Boyd, 1932a, b; Ewing, 1949)
	7		Type T, Lavington I, *"S. etousae"* (Ewing, 1946)
	8		Serotype 112 (Cox and Wallace, 1948)
	9		Serotype 1296/7 (Boyd, 1946; Ewing et al., 1951)
	10		Serotype 430 (Ewing and Taylor, 1951) and D15 (Szturm et al., 1950)
	11		Serotype 34 (Ewing and Taylor, 1951)
	12		Serotype 123 (and "M") (Ewing and Hucks, 1952)
	13		Serotype 425 (Ewing and Hucks, 1952)
	14		Serotype 2770-51 (Ewing and Hucks, 1952)
	15		Serotype 703 (Ewing et al., 1952a)
	16		Serotype 2710-54 (Ewing et al., 1958)
	17		Serotype 3615-53 (Ewing et al., 1958)
	18		Serotype E10163 (1344-78) (Gross et al., 1980)
	19		Serotype E16553 (Gross et al., 1982)
Subgroup D			
S. sonnei			Sonne-Duval, Sonne III, Kruse E, *"S. ceylonensis"* A (Duval, 1904; Sonne, 1915)

[a]The serologic subdivision of serotypes is done for only *S. flexneri*. The roman numerals designate the "type" or serotype-specific antigen and the Arabic numerals designate group antigens that are shared among members of the different types. Different subserotypes are defined by the combination of group antigens they possess. Group antigens shown in parentheses may be expressed in varying amounts (undetectable to strongly positive) by members of certain subserotypes. For the reactions of *S. flexneri* in typing antisera see Ewing (1986c). For *S. flexneri* serotype 3, only two subserotypes are currently recognized; strains previously designated as subserotype 3c are now identified as subserotype 3b [III:(3,4), 6] (Petrovskaya and Khomenko, 1979; Brenner, 1984b).

lipopolysaccharide (LPS) molecule. For *S. sonnei*, colonies of freshly isolated strains with a smooth appearance are termed form I and are usually virulent. Form I colonies are genetically unstable and dissociate to rough-appearing colonies termed form II, which lack 2-amino-deoxy-L-altruonic acid from their LPS O-repeating unit (Kontrohr, 1977). This form variation in *S. sonnei* is associated with the loss of the 180–220 kilobase pairs (kbp) invasion plasmid. Colonial variation, which also correlates with the presence of the invasion plasmid, has been described for colonies growing on agar containing the dye Congo red. Smooth colonies that appear red on this agar are virulent, while colorless colonies are avirulent (Maurelli et al., 1984a). The dye interacts

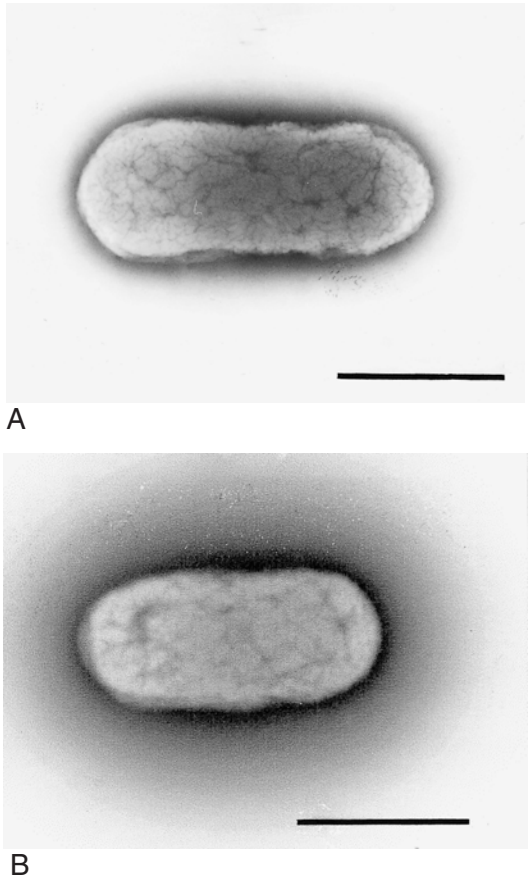


FIGURE BXII.γ.209. Electron micrograph of (*A*) *S. dysenteriae* 1 strain A5468 and (*B*) *S. flexneri* 2a strain 3342-87 prepared by negatively staining in 0.5% uranyl acetate. Bar = 1000 nm. (Micrograph courtesy of Charles D. Humphrey, CDC.)

with outer membranes and outer membrane proteins, but not with lipopolysaccharides. Only smooth colonies are scored for virulence using this dye because rough colonies, which are avirulent, can nonspecifically bind the dye, presumably by exposure of non-virulence-associated outer membrane proteins.

Nutrition and growth conditions The shigellae are aerobes and facultative anaerobes. Their optimal growth temperature is about 37°C. They generally grow less rapidly than most strains of *E. coli* and other members of the family *Enterobacteriaceae*. The average interval between cell divisions of *S. dysenteriae* in milk was reported to be 23 min compared with 12.5 min for *E. coli* (Sinclair, 1972). Ahmed et al. (1988) studied the nutritional requirements of 375 clinical isolates from the four species of *Shigella* for growth in a minimal medium containing glucose, ammonium sulfate, and inorganic salts and observed that most isolates would not grow in this medium. They found that virtually all isolates that failed to grow in the minimal medium could grow if the medium was supplemented with methionine, nicotinic acid, and tryptophan. Methionine and tryptophan appeared to be an obligatory requirement for *S. dysenteriae* 1 strains, while the combination of nicotinic acid and tryptophan was required for *S. dysenteriae* 2 strains.

In contrast to *Salmonella*, *Shigella* species and certain isolates of *E. coli* are acid-resistant (Gordon and Small, 1993; Small et al., 1994). Stationary phase cells of *S. flexneri* have the ability to survive for several hours at pH 2.5, which is hypothesized to contribute to their low infective dose by allowing them to safely pass through the stomach before colonizing the intestinal tract. The ability to survive exposure to acid is a complex phenotype, which depends on growth phase, media, and possibly a variety of proteins located at different subcellular locations. At least three genes, *hdeA*, *rpoS*, and *gadC*, play a role in mediating the acid-resistance phenotype under various conditions (Waterman and Small, 1996).

Metabolism and metabolic pathways Like *E. coli*, shigellae are chemoorganotrophic and have both a respiratory and a fermentative type of metabolism. They are generally less metabolically active than typical *E. coli* strains. They produce pyruvate from the fermentation of glucose and other sugars and convert it primarily by mixed acid (formic acid) fermentation to formic acid, acetic acid, and ethanol. In contrast to *E. coli* strains, shigellae do not produce gas from the fermentation of sugars (anaerogenic), because they lack the complex hydrogenlyase system (formic hydrogenlyase) that splits formic acid into equal amounts of CO_2 and H_2. Exceptions include some strains of *S. flexneri* serotype 6 and *S. boydii* serotypes 13 and 14. In addition to the traits described above, members of the genus *Shigella* do not hydrolyze urea and do not produce detectable amounts of acetoin (acetyl methyl carbinol) or 2,3-butyleneglycol (Voges–Proskauer test negative). They also do not produce phenylalanine deaminase and do not produce acid from α-D-methylglucoside, erythritol, or esculin. They are uniformly negative for lysine decarboxylase. The pathogenic and evolutionary significance of this negative phenotype is addressed below in the pathogenicity section.

Shigella strains vary in their ability to metabolize glycerol. A study characterizing the glycerol dehydrogenase activity among *Shigella* species showed that the type of glycerol dehydrogenase produced could be used to help differentiate *Shigella* species from *E. coli* (Bouvet et al., 1995b). In this study, no strain of *Shigella*, with the exception of the Manchester biotype of *S. flexneri* serotype 6, was found to produce an NAD^+-linked glycerol dehydrogenase, glyDH-II, while the majority of *E. coli* strains contained this enzyme.

Genetics Extensive genetic homology between *Shigella* and *E. coli* has permitted the use of classical genetic techniques, such as conjugal transfer and integration of chromosomal material between *E. coli* and *Shigella* (Luria and Burrous, 1957; Falkow et al., 1963; Formal et al., 1970, 1971; Sansonetti et al., 1983) and the generation of R plasmids (Timmis et al., 1985), to construct maps of the *Shigella* chromosome based on the known map for *E. coli* K-12. A *Not*I restriction map of the chromosome of *S. flexneri* 2a, with the assignment of nine virulence-associated loci identified by Tn5 insertions, was constructed by Okada et al. (1991). Genes that play a role in pathogenesis have received extensive study and were reviewed by Parsot and Sansonetti (1996), and are highlighted below in the pathogenicity section. Genes involved in O-antigen synthesis have also received considerable attention and are reviewed by Brahmbhatt et al. (1992) and discussed below in the antigenic structure section.

The work of Brenner et al. (1972a, 1973a) clearly established the genetic basis for the close biochemical and serologic relatedness between *E. coli* and *Shigella* that had been appreciated for many years. Their findings from DNA reassociation reactions followed by thermal elution chromatography on hydroxyapatite showed that, with the exception of *S. boydii* 13 strains, strains of

Shigella from all four species were as related to each other (sharing 80% or more of their nucleotide sequences) as they were to strains of *E. coli* (Brenner et al., 1973a). Estimates of genome size by I-*Ceu*I macrorestriction analysis revealed that the chromosome sizes of all four *Shigella* species were in a similar range to those observed for *E. coli* (Shu et al., 2000). By this analysis, the genome sizes for the type strains of the four species of *Shigella* were 4.415×10^6 bp for *S. dysenteriae*, 4.792×10^6 bp for *S. flexneri*, 4.645×10^6 bp for *S. boydii*, and 4.501×10^6 bp for *S. sonnei*, while those for *E. coli* were 4.653×10^6 bp and 4.816×10^6 bp for two *E. coli* strains (Shu et al., 2000). The genome sequences of two different isolates of *S. flexneri* 2a have been published and are deposited in GenBank under accession numbers AE014073 and AE005674 (Jin et al. (2002); Wei et al., 2003).

Published ribosomal RNA sequences for the four species of *Shigella* and of *E. coli* are nearly identical, with more than 99% homology (Shu et al., 2000). Like *E. coli*, *Shigella* strains have seven ribosomal RNA operons as determined by restriction analysis with I-*Ceu*I, an enzyme that specifically cuts a 26-bp site in the 23S rDNA sequence in *rrn* operons (Shu et al., 2000). The mapping of seven *E. coli* genes, each known to reside on a different I-*Ceu*I fragment in *E. coli* K-12, revealed that some chromosomal rearrangements involving the fragments corresponding to fragments D and E of *E. coli* K-12 took place in *S. dysenteriae* and *S. flexneri*. Available findings did not permit a determination of the mechanism (translocation versus inversion of fragments) for these rearrangements. Hybridization patterns of *S. boydii* and *S. sonnei* strains were similar to those observed for *E. coli* strains, while the patterns for the *S. dysenteriae* and *S. flexneri* strains were each distinct.

Shigellae can carry a variety of insertion sequences (IS elements) including IS*1* (Nyman et al., 1981; Kharat and Mahadevan, 2000); iso-IS*1* (Ohtsubo et al., 1981); IS *2* (Soldati and Piffaretti, 1991); IS*5* (Schoner and Schoner, 1981); IS*200* (Gibert et al., 1990); IS *630* (Houng and Venkatesan, 1998); and IS*911* (Prere et al., 1990). IS elements comprise over 6% of the sequenced genome of *S. flexneri* 2a; 314 IS elements were identified in strain 301 and 284 IS elements in strain 2457T (Jin et al. (2002); Wei et al., 2003). Some of these, such as IS *1* and iso-IS*1*, are present in large numbers in the chromosome of *Shigella* (50 to >150 copies per chromosome, respectively) and have been associated with negative phenotypes. For example, the inability of *S. sonnei* to utilize the β-glucoside salicin has been shown to involve the insertional inactivation of the gene *bglB*, encoding phospho-β-glucosidase B, by a novel IS (Kharat and Mahadevan, 2000). In contrast to adverse effects of IS elements on some genes, the IS *630* element appears to be required for the stable expression of form I antigen in *S. sonnei* (Houng and Venkatesan, 1998).

Plasmids, phages and phage typing, bacteriocins Shigellae carry a variety of plasmids. The most notable is the large (180–220 kbp) invasion plasmid, which belongs to the RepFIIA family of replicons (Silva et al., 1988) and is present in all virulent strains of *Shigella*. This plasmid carries genes that play an essential role in these organisms' ability to cause invasive disease and appears to contain regions from different origins (mol% G + C values ranging from 30% for *virF* to 49% for *sepA*). The complete DNA sequence of the virulence plasmid of *S. flexneri* has been determined (Buchrieser et al., 2000; Venkatesan et al., 2001). The organization and origin of the genes encoded on the plasmid are discussed in these articles. In some serotypes, plasmids carry genes involved in synthesis of the O antigen (Kopecko et al., 1980; Watanabe and Timmis, 1984). Antibiotic resistance plasmids are also common among the shigellae. Because of the variety of plasmids carried by *Shigella*, plasmid profile analysis has been used widely to discriminate between strains during outbreak investigations. The use of this technique has declined in recent years in favor of molecular methods that examine differences in total cellular DNA and are less influenced by environmental or host pressures that can result in loss or acquisition of plasmids.

Shigellae have also been reported to carry Hsd (host specificity for DNA) plasmids. Strains of *S. flexneri* and *S. sonnei* carrying plasmid-encoded isoschizomers of either *Eco*RII or *Nci*I (Lee et al., 1997) and a strain of *S. boydii* serotype 13 carrying a plasmid-encoded isoschizomer of *Nru*I (Mise et al., 1986) have been described.

Shigellae are susceptible to a variety of bacteriophages, and this property has permitted the development of several phage typing systems (Thomen and Frobisher, 1945; Hammarström, 1947, 1949; Slopek et al., 1968, 1973; Lazlo et al., 1973; Pruneda and Farmer, 1977). A detailed description of the methods and the different bacteriophages used for typing *Shigella* was reported by Bergan (1979). Systems described to date, which typically use a panel of 10–20 bacteriophages to detect different patterns of lysis on a test strain, have focused on discriminating between strains within a serogroup or serotype. Discrimination between strains is generally good; for example, the method described by Lazlo et al. (1973) identified 90 types among more that 4000 strains of *S. flexneri* tested, and that of Pruneda and Farmer (1977) discriminated 87 different types among 265 strains of *S. sonnei*. Control of assay conditions, particularly the media and the smoothness of the cultures, is important for obtaining reproducible results. While the majority of strains can be characterized by phage typing, common phage types exist and it may be necessary to apply additional typing methods to achieve adequate discrimination.

Genes carried by some bacteriophages in shigellae have been identified. Among the serotypes of *S. flexneri*, bacteriophages have been demonstrated to carry genes, such as acetyltransferase, glucosyltransferase, or novel O-antigen polymerase genes, that play a role in determining the type and group determinants of the O antigens (Gemski et al., 1975; Lindberg et al., 1978; Clark et al., 1991a; Huan et al., 1997a, b; Mavris et al., 1997; Guan et al., 1999). Because many bacteriophages use O-antigenic polysaccharide chains as receptors for adsorption and infection, the modification of the host bacterial O antigens by the incoming phages is hypothesized to be a protective mechanism for excluding homologous phages from entering and may also help lysogenized cells evade preexisting host immunity.

Findings reported by McDonough and Butterton (1999) provide evidence, in the sequences surrounding the Shiga toxin gene, suggesting that *S. dysenteriae* 1 was once lysogenized by a Shiga toxin-encoding lambdoid prophage, which became defective as a result of deletions after IS element insertions and rearrangements. These authors also provided evidence that Shiga toxin-encoding phages from *E. coli* can be stably introduced into *Shigella*. Other than *S. dysenteriae* 1, Shiga toxin-producing strains of *Shigella* are extremely rare.

Antigenic structure The different serotypes are distinguished by antigenic determinants that reside in the O antigen, which is part of the LPS molecule. The LPS molecule consists of three

parts: lipid A, which is made up of sugars and fatty acids and anchors the LPS molecule in the outer membrane; the core, which is made up of a single sequence of heptoses and hexoses and links the lipid A to the O antigen; and the O-antigen chain, which is composed of a repetitive sequence of hexoses and extends from the surface of the bacterium. The genetics of O antigen biosynthesis have been extensively studied and are reviewed by Schnaitman and Klena (1993) and Whitfield (1995). O antigens may contain from 10 to as many as 30 repeats of an oligosaccharide unit (O unit) each composed of three to six sugars. The diversity of O antigens is a result of varying combinations of sugars in the O unit, the type of chemical linkages between them, and the presence of nonsugar moieties such as O-acetyl residues or amino acids. Six to nineteen genes may be involved in the synthesis of the O antigen, and these are typically located together on the chromosome in a region (10 kbp or greater) called the *rfb* cluster. Depending on the sugars making up the O unit, serotypes may have completely different sets of genes. For certain shigellae, such as *S. dysenteriae* 1, *S. sonnei*, and *S. flexneri* 1–5, one or more genes for the synthesis of the O unit are carried on plasmids or lysogenic bacteriophages. Like *E. coli* and other Gram-negative bacteria, *Shigella* have a conserved 39-bp sequence, designated JUMPstart for "just upstream of many polysaccharide-associated gene starts," located in the noncoding region upstream of the *rfb* cluster (Hobbs and Reeves, 1994). This region has been used to develop a molecular method for serotyping *Shigella* isolates (Coimbra et al., 1999).

The O antigens of most serotypes of *Shigella* are identical or partially related to the O antigens of *E. coli* (Ewing, 1953, 1986c). At least 13 identical and numerous reciprocal serologic relationships exist between *Shigella* and *E. coli* (Ewing, 1986c).

With the acceptance of provisional serotypes E22383 and E23507 as new serotypes (Ansaruzzaman et al., 1995), *S. dysenteriae* contains 15 serotypes, each with a distinctive antigen by which it can be recognized; there are few cross-reactions, either within the species or with other species that present difficulties for serotyping strains. Of the different serotypes within this species, the O antigen of *S. dysenteriae* 1 has been the most extensively studied because of efforts to develop LPS-based antidysentery vaccines. Genes involved in the synthesis of its O antigen have been identified, and their structure and function were reviewed by Schnaitman and Klena (1993). Eight of these genes are located together in the chromosome and two genes (*rfp* and *rfe*) lie outside the chromosomal cluster; *rfp* is carried on a 9-kbp multicopy plasmid.

S. flexneri contains eight serotypes and nine subserotypes. The serotypes are antigenically related, but each has a qualitatively distinct major (type) antigen; the group antigens are shared by other members of the species. Because of the important intragroup relations, highly absorbed sera are needed for the detailed serotyping of *S. flexneri*. The immunochemical and genetic bases of the complex antigenic structures of the species have been summarized by Petrovskaya and Bondarenko (1977). The O antigens of all serotypes, except *S. flexneri* 6, contain group antigens 3, 4 as a main primary structure. The type-specific antigens I, II, IV, and V and the group antigens 7, 8 are all the result of phage conversion of the 3, 4 antigens resulting in the incorporation of α-glycosyl and/or glucosyl groups to the common O-repeating unit of the LPS molecule. Type-specific antigen III and group antigen 6 differ from the above antigens in that they contain acetyl groups. Nevertheless, these antigens are also formed as a result of phage conversion of the 3, 4 antigens. The genes involved in synthesis of the common O-repeating unit are located on the chromosome in the *rfb* cluster.

The classification of *S. flexneri* 6 as a serotype of *S. flexneri* was questioned by Petrovskaya and Bondarenko (1977), who proposed that it be transferred to *S. boydii* because the immunochemical structure and genetics of its O antigen were more consistent with those found among serotypes of *S. boydii* than among serotypes 1-5, Y and X of *S. flexneri*. In 1984, the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Enterobacteriaceae* considered this proposal and upheld the 1973 recommendation of the Subcommittee Working Group on *Shigella* to reject reclassification of *S. flexneri* 6 as a serotype of *S. boydii* on the grounds that it would have no practical value and would cause confusion by altering a well-accepted classification system (Brenner, 1984b).

S. boydii contains 19 serotypes and each has a qualitatively distinct antigen; there may be some cross-reactions with antisera to other *Shigella* species, but these seldom interfere with diagnosis. Serotypes 10 and 11 share a major antigen, although each possesses a specific antigen.

S. sonnei contains only one serotype but may exist in one of two forms or "phases": form I (smooth colonial appearance) and form II (rough colonial appearance). Each form has a distinctive antigen, and antiserum containing agglutinins for both phases should be used for identification. Form I cells, which are virulent and carry the 180-kbp invasion plasmid, are typically isolated from acutely ill individuals. These cells can rapidly and irreversibly switch to form II cells, which are avirulent, when the invasion plasmid is lost. Form II cells may be isolated from ill individuals; however, they are more frequently isolated from individuals during convalescence and toward the end of an outbreak.

The O antigen unit of form I cells is structurally unique compared with those from other shigellae or *E. coli*, and it is chemically identical to that found in serotype 17 of *Plesiomonas shigelloides* (Rauss et al., 1970; Kenne et al., 1980). With the exception of two (*wzz* and *wbg*), the genes responsible for expression of the form I O antigen are located on the invasion plasmid and are nearly identical to those present in *P. shigelloides* (Shepherd et al., 2000). Factors influencing the loss of the plasmid carrying these genes are not well understood; however, Houng and Venkatesan (1998) propose that an insertion element present in the plasmid may be necessary for the stable expression of the form I antigen. These authors discovered the insertion element IS *630* within the O antigen gene cluster of all virulent strains of *S. sonnei* and observed that recombinant strains of *E. coli* carrying the cloned *S. sonnei* O antigen genes could stably express the form I antigen only when the IS *630* element was present.

While the form I O antigen expressed by *S. sonnei* is encoded by the plasmid-borne O antigen genes from *P. shigelloides* (Shepherd et al., 2000), findings by Lai et al. (1998) show that *S. sonnei* once had a typical chromosomal O antigen gene cluster. The remnant O antigen gene cluster apparently arose from a deletion due to homologous recombination between *manB* genes present in the adjacent O antigen and colanic acid gene clusters. The remnant chromosomal O antigen genes are not functional.

In addition to the recognized serotypes of shigellae, a number of provisional *Shigella* serotypes have been described. These may be added to the serotyping schema in the future, but in the meantime they remain *sub judice*, and antisera for their identification are usually available only at a very few reference laboratories. Provisional serotypes under consideration at present include serotypes E670/74 (Gross et al., 1989), 3162-96 (Kuijper

et al., 1997), 93-119 (Matsushita et al., 1997), 96-204 (Matsushita et al., 1998), and 96-265 (Frank Rogers, National Laboratory for Enteric Pathogens, Health Canada, Winnipeg, Canada; personal communication), which are biochemically consistent with members of subgroup A; serotypes Y394 (Wehler and Carlin, 1988), 88-893 (Matsushita et al., 1992a), 89-141 (Matsushita et al., 1992b), which are biochemically and antigenically consistent with members of subgroup B; and serotypes 1621-54 (Ewing et al., 1958), E28938 (Gross et al., 1989), and 99-4528 (Frank Rogers, National Laboratory for Enteric Pathogens, Health Canada, Winnipeg, Canada; personal communication), which are biochemically consistent with members of subgroup C.

Pathogenicity Shigellae are pathogens of humans and other primates. Although there have been occasional reports of infections in dogs, other animals are resistant to infection. Laboratory animals such as mice, rabbits, and guinea pigs may be infected orally but only following starvation and treatment with gastric antacids and antiperistaltic agents.

Shigellosis (bacillary dysentery) is transmitted orally through contaminated food and water or by direct fecal-oral spread. A review of the literature estimated the annual number of cases throughout the world to be 164.7 million, of which 163.2 million were in developing countries (with 1.1 million deaths). A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involved children less than 5 years of age (Kotloff et al., 1999). Studies of volunteers have demonstrated that ingestion of as few as 200 organisms is sufficient to cause dysentery (DuPont et al., 1989). The incubation period is from 1 to 7 days with symptoms commonly manifesting on day 3. Shigellosis often (but not always) begins with a watery diarrhea that precedes the characteristic dysentery symptoms. The diarrhea phase probably results from production of enterotoxins by the bacteria as they transit through the small intestine.

The lesions of bacillary dysentery are usually restricted to the rectum and large intestine, but in severe cases part of the terminal ileum may be affected. *Shigella* penetrates into the epithelial cells lining the colon, multiplies within these cells, and spreads from cell to cell through the mucosa. The foci of infected cells coalesce to form abscesses. Small volume bloody and mucoid stools contain dead cells along with mucus and large numbers of bacteria. Typically there is acute inflammation with ulceration of the epithelium, and the presence of polymorphonuclear leukocytes in the stools is consistent with the inflammatory nature of shigellosis. Common clinical signs include fever, severe abdominal pain, and cramping. The most severe forms of shigellosis are caused by strains of *S. dysenteriae* 1, which also produces a potent cytotoxin (Shiga toxin) that has been shown to play a role in the severity of the illness it causes (Fontaine et al., 1988). Shiga toxin-producing strains can cause hemolytic uremic syndrome. *S. sonnei* strains cause milder forms of the disease, while *S. flexneri* and *S. boydii* strains can cause either severe or mild illness. Bacillary dysentery is a self-limiting disease. The organisms rarely spread deeper than the lamina propria, and bloodstream involvement is uncommon.

Treatment of shigellosis with appropriate antimicrobial therapy results in decreased duration of both symptoms and excretion of the organism (Haltalin et al., 1967; Salam and Bennish, 1991); however, empirical antimicrobial therapy for this illness has become complicated by the increasing incidence of multiple drug resistance among *Shigella* strains. Of historical note, the first observation of multiple, transferable drug resistance in bacteria was in *Shigella* in Japan (Ochiai et al., 1959). Over the years, *Shigella* species have become increasingly resistant to most of the widely used antibiotics (Bennish et al., 1992). Multiple antibiotic resistance in *S. dysenteriae* 1 is so widespread that it is uniformly susceptible only to the fluoroquinolones (Sack et al., 1997). Antimicrobial resistance surveys of *Shigella* species conducted during the 1990s in developed and developing countries showed that multiple drug resistance exists in strains of all species (Keusch and Bennish, 1998; Mates et al., 2000; Replogle et al., 2000). The resistance patterns varied by species and by country, highlighting the need for monitoring of local antimicrobial resistance patterns to develop appropriate empirical therapy. Because *Shigella* infections are typically self-limiting and because antibiotic resistance frequently develops after treatment, some have proposed that antimicrobial therapy be reserved for the most severely ill patients, immunocompromised patients, and patients for whom eliminating carriage is a public health priority (Weissman et al., 1973; Replogle et al., 2000). Others feel that, because the infection is generally transmitted from person to person and the infected person represents the major reservoir of infection, each patient with a positive stool culture or with known bacillary dysentery should be treated to prevent the spread of infection (Dupont, 2000).

The hallmarks of *Shigella* pathogenicity are induction of diarrhea, the ability to invade eucaryotic cells, multiplication inside these cells, and spread from cell to cell. The rabbit ileal loop assay has been used as an experimental model to demonstrate production of at least two different enterotoxins by *S. flexneri*, which may play a role in the diarrhea caused by the organism (Fasano et al., 1995; Nataro et al., 1995). The invasive properties of *Shigella* have been demonstrated in experimental infection of monkeys (LaBrec et al., 1964) as well as in the Serény test, which measures the ability of the bacteria to invade and produce keratoconjunctivitis in the guinea pig eye (Sérèny, 1957). Invasion can also be demonstrated in mammalian cells in tissue culture (LaBrec et al., 1964; Ogawa et al., 1967; Day et al., 1981). The ability of the bacteria to spread from cell to cell after invasion is measured by the plaque assay (Oaks et al., 1985). While shigellae are killed after being taken up by polymorphonuclear leukocytes (Mandic-Mulec et al., 1997), they induce apoptosis in macrophages and kill these cells after uptake (Zychlinsky et al., 1992). *S. dysenteriae* serotype 1 produces Shiga toxin, a potent inhibitor of eucaryotic protein synthesis (O'Brien et al., 1992). Shiga-like toxins are also produced by enterohemorrhagic strains of *E. coli* (O'Brien et al., 1984).

Shigella strains invade the intestinal mucosal surface via a pathogen-directed phagocytic process that actively involves elements of the host cytoskeleton (Tran Van Nhieu et al., 2000). Unlike most other intracellular bacterial pathogens, however, *Shigella* strains rapidly lyse the endocytic vacuole upon entry and are released free into the host cell cytoplasm where they replicate. Shigellae are actively motile during growth inside the host cell. This motility is unusual in that it is not driven by bacterial-based flagella. Rather a bacterial protein, expressed in the outer membrane at one pole of the bacterium, catalyzes the polymerization of host cell actin filaments (Makino et al., 1986; Bernardini et al., 1989). Formation of actin tracks literally propels the bacterium through the cytoplasm as actin monomers polymerize into long filaments from one pole of the bacterium. This motility is required for cell-to-cell spread of *Shigella*. Mutant strains of *Shigella* that fail to display this intracellular motility produce a greatly attenuated form of disease in the monkey model, thus confirm-

ing this phenotype as a critical hallmark of *Shigella* pathogenesis (Sansonetti et al., 1991).

Virulence in *Shigella* spp. is dependent on expression of a group of genes encoded on a large plasmid. The role of this plasmid in *Shigella* virulence was first demonstrated in *S. flexneri* and *S. sonnei* (Sansonetti et al., 1981, 1982). Subsequently, all virulent species of *Shigella* and enteroinvasive strains of *E. coli* were shown to carry a 180–220-kbp plasmid that shares substantial DNA homology with these prototype virulence plasmids (Sansonetti et al., 1985). Introduction of these plasmids into plasmid-cured strains of *Shigella* or laboratory strains of *E. coli* K-12 imparts on the bacteria the ability to invade mammalian cells in tissue culture. Thus, the *Shigella* virulence plasmid encodes all the genes required for the invasive phenotype of *Shigella*. The DNA sequence of the virulence plasmid of *S. flexneri* 5a has been determined and deposited in GenBank under accession numbers AF348706, AL391753, and AF386526 (Buchrieser et al., 2000; Venkatesan et al., 2001; Jin et al. (2002)).

A 37-kbp region of the plasmid contains the minimal sequence needed for invasion (Maurelli et al., 1985; Baudry et al., 1987). The genes in this region are roughly grouped into two clusters transcribed from opposite strands. They encode the Ipa proteins, required for inducing uptake of the bacteria by mammalian cells, and Mxi/Spa proteins of a dedicated type III secretion apparatus for transport of the Ipa proteins outside the bacteria. Type III secretion systems are specialized transport systems found in animal and plant pathogens and are responsible for mediating interactions between the bacterial pathogen and its eucaryotic host (Cornelis and Van Gijsegem, 2000). The Mxi and Spa proteins of the Shigella type III secretion system show strong homologies with the analogous proteins of the type III secretion systems of *Salmonella* Typhimurium, enteropathogenic and enterohemorrhagic *E. coli*, and *Yersinia* spp. The *Shigella ipa* genes (*ipaBCDA*) encode the secreted effector molecules that mediate the bacteria-induced phagocytic (invasion) event. The Ipa proteins (invasion plasmid antigens) are the immunodominant antigens recognized by convalescent-phase sera from shigellosis patients and challenged monkeys (Maurelli et al., 1985; Oaks et al., 1986). Unlike the genes of the virulence plasmid that are involved in invasion and postinvasion steps, the chromosomal genes associated with virulence of *Shigella* appear to mostly encode modulators of pathogenicity.

Of interest from the point of view of bacterial evolution and pathogenesis are the genes that are present in nonpathogenic strains of *E. coli* but are missing from the chromosome of *Shigella*. A cryptic prophage in the *E. coli* K-12 chromosome that is missing in shigellae encodes an outer membrane protease, OmpT. When the *ompT* gene is introduced into *Shigella* by conjugation, the strain remains invasive but loses the ability to spread from cell to cell. This phenotype is due to OmpT protease degradation of IcsA, the bacterial protein responsible for actin polymerization and motility (Nakata et al., 1993). Another example of a missing genetic locus in the *Shigella* genome is *cadA*, the gene for lysine decarboxylase. Although lysine decarboxylase activity is present in >85% of *E. coli* strains, it is absent in all strains of *Shigella* spp. and enteroinvasive *E. coli*. When *cadA* is reintroduced into *S. flexneri*, the decarboxylation of lysine generates cadaverine. The *Shigella* enterotoxins are inhibited by cadaverine (Maurelli et al., 1998). Thus, these genes can be considered antivirulence genes and the missing genetic locus, a black hole. The example of *Shigella* illustrates another way that pathogens evolve from their nonpathogenic commensal relatives by both acquiring genes (e.g., the virulence plasmid) that contribute to virulence and deleting genes that are incompatible with expression of these new virulence traits.

Growth temperature is the critical stimulus for regulation of virulence in *Shigella*. Wild-type *Shigella* strains grown at 37°C display all the attributes of virulence, whereas the same strains grown at 30°C are avirulent in the Serény test and fail to invade tissue culture cells (Maurelli et al., 1984b). The loss of virulence is reversible and the organisms regain full virulence (invasion and ability to provoke keratoconjunctivitis) after the growth temperature is shifted to 37°C. Paradoxically, growth of *Shigella* at 37°C in the laboratory leads to spontaneous deletion or loss of the virulence plasmid. Evidence suggests that expression of virulence genes *in vitro* is not well tolerated by the bacteria and leads to selection of variants that no longer express these genes either due to mutations in regulatory genes or deletion of plasmid sequences (Schuch and Maurelli, 1997). Strains grown at 30°C retain the virulence plasmid intact.

Despite many years of effort, an effective vaccine against shigellosis still has not been developed. Formulations have included killed whole cells, live attenuated strains, subunit vaccines (conjugate and proteosome), and ribosomal vaccines. The World Health Organization has placed *Shigella* vaccine development on its list of priorities for the Global Program for Vaccines and Immunization (WHO, 1997).

Ecology Shigellae are host-adapted to humans and subhuman primates, where they typically inhabit the intestinal tract. *Shigella* infections in captive primates are not uncommon; however, two groups of investigators (Takasaka et al., 1964; Carpenter and Cooke, 1965) found no evidence that wild primates living without human contact are infected. During the acute stage of the illness, shigellae are typically shed in large numbers in the feces. The number of organisms dramatically declines during recovery, and in some individuals a carrier state may develop in which organisms may persist in the feces for several weeks after the symptoms have subsided. The spread of *Shigella* infection is typically from person to person by fecal–oral transmission or from contaminated food or water. In situations without good sewage disposal and poor hygiene conditions, flies may act as vectors (Levine and Levine, 1991).

Enrichment and Isolation Procedures

Food and water The minimum infecting dose of shigellae is small (10–100 organisms), and occurrence of the organisms in food, milk, and water may be significant even when only a small number of organisms are present. There are no reliable and effective enrichment methods, however, and the true incidence of *Shigella* contamination of foodstuffs cannot be accurately determined. The GN (Gram-negative) broth of Hajna (1955) may be useful for enrichment of *Shigella*, and it is recommended that the investigation of foodstuffs include an enrichment step using this medium. Subsequent steps in the isolation of *Shigella* from foods should follow the procedure recommended for fecal specimens.

Fecal specimens Freshly passed stools should be examined, although, if this is not possible, fecal swabs or rectal swabs showing marked fecal staining may be used. The specimens should be collected during the acute stage of the disease and before any chemotherapy is started. Specimens should be examined as soon after collection as possible. If the specimen includes blood and mucus, these should be included in the portion examined.

Although GN broth and Selenite broth are commonly used, no enrichment medium for *Shigella* consistently improves the

recovery rate beyond that obtained by direct plating alone. Because some strains of *Shigella* grow poorly on inhibitory media, both a relatively noninhibitory selective medium (e.g., MacConkey) and an inhibitory medium (e.g., xylose desoxycholate, deoxycholate citrate agar, or Hektoen enteric agar) should be used for isolation. Salmonella–Shigella (SS) agar is not recommended because it inhibits the growth of some strains of *S. dysenteriae* 1. Instructions for preparation of these media are given by Ewing (1986d) and Atlas (1997). Specimens are streaked onto the chosen media, and after overnight incubation at 35–37°C, lactose- or xylose-nonfermenting colonies are selected for further examination. Even when stool specimens from acute dysentery are examined, there may be only a scanty growth of *Shigella*. Suspicious colonies should be tested by biochemical and serologic methods (direct slide agglutination or coagglutination of antibody-coated latex beads) to confirm the identification of shigellae. For *S. flexneri*, which is the most difficult to prepare typing antisera against because of its shared group antigens, a panel of monoclonal antibodies has been developed for typing (Carlin et al., 1989). In some reference laboratories, molecular methods, such as DNA hybridization (Boileau et al., 1984), PCR (Frankel et al., 1990; Sethabutr et al., 1993; Yavzori et al., 1994; Houng et al., 1997; Villalobo and Torres, 1998; Sethabutr et al., 2000), enzyme-linked immunosorbent assays (Floderus et al., 1995; Pal et al., 1997), and immunomagnetic capture assays (Islam et al., 1993), targeting O antigens or invasion-associated genes/proteins are being used to detect shigellae and enteroinvasive *E. coli*.

Laboratory surveillance for *Shigella* infections is conducted in many countries and has been valuable for investigating outbreaks of shigellosis and for guiding vaccine development efforts. Serotyping has been and continues to be the primary method for discriminating between strains. During outbreaks or for highly prevalent serotypes such as *S. sonnei*, isolates are often subjected to a variety of additional phenotypic or molecular typing methods. Virtually all the secondary typing methods are predicated upon knowledge of the isolate's serotype. Other methods commonly used for differentiating between *Shigella* strains include colicin typing and phage typing. The colicin typing scheme for *S. sonnei* described by Abbot and Shannon (1958) has been used widely and distinguishes 14 types using 15 indicator strains (see Procedures for Testing Special Characters). Phage-typing schemes have also been used by many investigators. While a number of schemes have been described for *S. flexneri* and *S. sonnei*, only a few have been reported for *S. dysenteriae* and *S. boydii* (reviewed by Bergan, 1979). A variety of molecular subtyping methods have been applied to *Shigella*, which include ribotyping (Hinojosa-Ahumada et al., 1991; Faruque et al., 1992; Nastasi et al., 1993), IS element typing (Soldati and Piffaretti, 1991), enterobacterial repetitive intergenic consensus (ERIC) sequence-based PCR (Liu et al., 1995a), randomly amplified polymorphic DNA (Bando et al., 1998), and macrorestriction endonuclease analysis with pulsed-field gel electrophoresis (PFGE) (Soldati and Piffaretti, 1991; Liu et al., 1995a; Litwin et al., 1997). Because of its discriminatory potential, PFGE is the method preferred by many investigators for molecular subtyping studies, particularly for epidemiologic investigations of *S. sonnei* infections. This method proved invaluable in the investigation of a protracted outbreak of *S. sonnei* infections among members of traditionally observant Jewish communities in North America (Sobel et al., 1998) and was critical in an investigation of geographically unrelated cases of *S. sonnei* infection, identifying parsley from a single farm as the vehicle of infection (Centers for Disease Control, 1999).

Maintenance Procedures

Shigella cultures are maintained best frozen in liquid nitrogen. Liquid culture medium, such as trypticase soy broth, containing 10–20% glycerol is commonly used as a freezing medium. Cultures of *Shigella* may also be lyophilized or maintained on blood agar base or Dorset egg medium (stabbed slants or deeps) at room temperature; however, rough and degraded variants frequently arise under these conditions.

Procedures for Testing Special Characters

For colicin typing of *S. sonnei*, the organism under investigation is inoculated heavily in a broad streak across a blood agar plate and incubated at 37°C for 24 h. The bacterial growth is then removed from the agar by scraping with a glass slide, and the organisms remaining are killed with chloroform. The 15 indicator strains are streaked onto the plate at right angles to the original line of growth. After further incubation for 8–12 h, the patterns of inhibition of growth of the indicator strains can be examined and compared with a key. It is important that controls be included in every batch of tests.

Differentiation of the genus *Shigella* from other genera

The biochemical identification of *Shigella* is complicated by the similarity of some strains of other genera. In particular, strains of *Hafnia alvei*, *Providencia* spp., *Aeromonas* spp., and atypical *E. coli* can cause difficulties. Advances in our knowledge of the genetics of microorganisms have allowed the development of new approaches for identifying and detecting bacteria. Molecular strategies have been developed to facilitate microbial identification. Spierings et al. (1993) described a PCR assay targeting DNA sequences that encode the hypervariable surface-exposed regions of the outer membrane protein PhoE, which shows a high degree of species specificity and can differentiate strains of *Shigella* and *E. coli* from strains of other enteric bacteria.

Nonlactose-fermenting or anaerogenic strains of *E. coli* are commonly quite difficult to differentiate from shigellae. Of particular interest are members of the so-called Alkalescens Dispar (A-D) group, which contains nonmotile, anaerogenic biotypes of *E. coli*. These are best differentiated from *Shigella* by means of the Christensen's citrate and lysine decarboxylase tests, in which *Shigella* is always negative. The members of the A-D group were divided into eight serogroups based on their O antigens (Frantzen, 1950), although most of these are identical with or closely related to *E. coli* antigens.

Taxonomic Comments

The genus *Shigella* consists of nonmotile organisms that conform to the definition of the family *Enterobacteriaceae* and have the biochemical and antigenic properties described above. Circumscription of the genus is problematic because of the close genetic relationship that exists between *Shigella* spp. and *E. coli*. There are no unique traits exclusively associated with *Shigella*. The negative phenotypes identified with *Shigella* spp. can occur in *E. coli*, particularly among the nonmotile, anaerogenic biotypes of *E. coli*, while positive phenotypes seen among typical *E. coli* isolates, such as lactose or sucrose fermentation and gas production from the fermentation of glucose, can occasionally occur in *Shigella*. Some *E. coli* strains (enteroinvasive *E. coli*) can also cause a dysentery-like illness, which is mediated by similar plasmid-encoded virulence genes as those possessed by *Shigella*.

Although pathogenicity (i.e., the ability to cause dysentery)

was not endorsed by the *Enterobacteriaceae* Subcommittee of the International Committee on Bacteriological Nomenclature for the classification of *Enterobacteriaceae* (Carpenter, 1963), it has influenced and continues to influence the classification of *Shigella* by virtue of the fact that only strains that are capable of causing dysentery receive consideration for addition to this group. Strains that do not cause dysentery but yield biochemical reactions consistent with those produced by *Shigella* are not considered for inclusion in this genus. The preservation of this association between bacteria classified as *Shigella* and a distinct form of diarrheal illness (dysentery) has sustained a functionally useful nomenclature that might otherwise have yielded over the years to persuasive findings that reveal a sufficiently close relationship between *Shigella* and *E. coli* to consider them the same species (Brenner et al., 1972a, 1973a).

The names adopted by Pupo et al. (2000), (*Escherichia coli* clone Dysenteriae, *Escherichia coli* clone Flexneri, *Escherichia coli* clone Boydii, and *Escherichia coli* clone Sonnei), reflect the well-documented natural relationships that exist between *Shigella* and *E. coli* and have merit from a phylogenetic perspective; however, their widespread adoption will happen only when it is important to users to communicate these relationships through the bacterial names. Until that time, the medically useful link between the genus epithet *Shigella* and shigellosis, the term for the disease caused by these bacteria, will sustain the current nomenclature.

Findings from numerous studies raise questions about the inclusion of *S. boydii* 13 strains in the genus *Shigella*. The DNA–DNA reassociation findings reported by Brenner et al. (1982c) show that *S. boydii* 13 strains were related to each other, but clearly separable from other *Shigella* and *E. coli* (average 65% relatedness). Findings from MLEE (Ochman et al., 1983; Pupo et al., 1997), nucleotide sequence analysis of housekeeping genes (Pupo et al., 2000), ribotyping (Rolland et al., 1998), and esterase electrophoretic polymorphisms (Goullet and Picard, 1987) also show a distant relationship between *S. boydii* 13 strains and other shigellae.

Further Reading

Centers for Disease Control and Prevention. 1999. Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera, CDC, Atlanta.

Dorman, C.J. and M.E. Porter. 1998. The *Shigella* virulence gene regulatory cascade: a paradigm of bacterial gene control mechanisms. Mol. Microbiol. *29*: 677–684.

Ewing, H.E. 1986. Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th Ed., Elsevier Science Publishing Co., Inc., New York.

Keusch, G.T. and M.L. Bennish. 1998. Shigellosis. *In* Evans and Brachman (Editors), Bacterial Infections of Humans. Epidemiology and Control, 3rd Ed., Plenum Medical Book Co., New York. pp. 631–656.

Menard, R., C. Dehio and P.J. Sansonetti. 1996. Bacterial entry into epithelial cells: the paradigm of *Shigella*. Trends Microbiol. *4*: 220–226.

Sansonetti, P. and A. Phalipon. 1996. Shigellosis: from molecular pathogenesis of infection to protective immunity and vaccine development. Res. Immunol. *147*: 595–602.

Differentiation of the species of the genus *Shigella*

Biochemical characteristics useful for differentiating the species of *Shigella* are listed in Table BXII.γ.275.

List of species of the genus Shigella

1. **Shigella dysenteriae** (Shiga 1898) Castellani and Chalmers 1919, 935, Epit. Spec. Cons. Opinion 11 of the Jud. Comm. 1954a, 149[AL] (*Bacillus dysenteriae* Shiga 1898, 817.)*
dys.en.te'ri.ae. Gr. n. *dysenteria* dysentery; M.L. gen. n. *dysenteriae* of dysentery.

   Colonies of serotype 1 often have a pinkish tinge on Leifson's deoxycholate citrate agar. Catalase is not produced by serotype 1, but is usually produced by strains of other serotypes. D-mannitol is not fermented. Dulcitol is fermented by strains of serotype 5. Gas from the fermentation of sugars is not produced. Indole is not produced by serotype 1, but is always produced by strains of serotype 2; strains of other serotypes vary in indole production. All the serotypes have, at one time or another, been known by other designations, and these are shown in Table BXII.γ.276. Members are intestinal pathogens of humans and subhuman primates, causing bacillary dysentery. Humans are the primary reservoir. A long-term carrier state occurs in a small percentage of cases (Levine et al., 1973). Serotype 1 causes more severe disease than other serotypes and produces a potent protein exotoxin (Shiga toxin), which plays a role in the severity of the disease attributable to this serotype and in the development of hemolytic uremic syndrome in some individuals. Large epidemics in developing countries are commonly caused by serotype 1. Disease caused by other serotypes may be mild or severe.

   The mol% G + C of the DNA is: 53 (chemical analysis; Laskin and Lechevalier (1981)).

   Type strain: ATCC 13313, CIP 57.28, NCTC 4837.

   GenBank accession number (16S rRNA): X96966.

   Additional Remarks: Neotype strain ATCC 13313 was designated by Judicial Commission, 1963.

2. **Shigella boydii** Ewing 1949, 634, Epit. Spec. Cons. Opinion 11 of the Jud. Comm. 1954a, 149[AL]*
boy'di.i. M.L. gen. n. *boydii* of Boyd; named after Sir John Boyd, a British bacteriologist.

   Catalase is produced. D-mannitol is fermented. Dulcitol is usually fermented by serotypes 2, 3, 4, 6, and 10, but this may be delayed. D-xylose fermentation is variable. Indole may or may not be produced. Gas-producing biotypes of *S. boydii* serotype 13 (Rowe et al., 1975) and serotype 14 (Carpenter, 1961) have been described. Members are intestinal pathogens of humans and subhuman primates, causing bacillary dysentery. Disease may be mild or severe. Humans are the primary reservoir. A long-term carrier state occurs in a small percentage of cases (Levine et al., 1973).

   The mol% G + C of the DNA is: not determined.

   Type strain: ATCC 8700, CIP 82.50, DSM 7532, NCTC 12985.

3. **Shigella flexneri** Castellani and Chalmers 1919, 937, Epit.

**Editorial Note:* This species is also known as subgroup A.

**Editorial Note:* This species is also known as subgroup C.

Spec. Cons. Opinion 11 of the Jud. Comm. 1954a, 149[AL]* *flex' ner.i.* M.L. gen. n. *flexneri* of Flexner, named after Simon Flexner, an American bacteriologist.

Catalase is produced. D-mannitol is fermented, except by biotype Newcastle, serotype 6 and a D-mannitol negative, D-xylose positive biotype of serotype 4a (sometimes known as *"S. rabaulensis"*). Dulcitol is fermented by certain biotypes of serotype 6 (see Table BXII.γ.275), some of which produce gas from fermentable sugars. Indole is not produced by serotype 6; in other serotypes indole production is variable. Members are intestinal pathogens of humans and subhuman primates, causing bacillary dysentery. Humans are the primary reservoir. Disease may be mild or severe. A long-term carrier state occurs in a small percentage of cases (Levine et al., 1973). Infection in individuals with the HLA-B27 histocompatibility antigen can be complicated by Reiter chronic arthritis syndrome.

The mol% G + C of the DNA is: 49 (chemical analysis) (Laskin and Lechevalier, 1981) and 50.9 (nucleotide sequence analysis) (Jin et al., 2002) and (Wei et al., 2003).

Type strain: ATCC 29903, CIP 82.48, DSM 4782.

GenBank accession number (16S rRNA): X96963; complete genome: AE005673 and AE014073.

4. **Shigella sonnei** (Levine 1920) Weldin 1927, 182, Epit. Spec. Cons. Opinion 11 of the Jud. Comm. 1954a, 149[AL] (*Bacterium sonnei* Levine 1920, 31.)*

son' ne.i. M.L. gen. n. *sonnei* of Sonne, named after Carl Sonne, a Danish bacteriologist.

On deoxycholate citrate agar colonies are at first colorless, but after a few days show bright pink papillae consisting of lactose-fermenting cells. On MacConkey agar, phase I colonies are indistinguishable from colonies of other shigellas, but phase II colonies are larger, flatter, and more translucent, and have an irregular edge. On subculture, phase I colonies produce both phase I and phase II colonies, but phase II colonies give rise to phase II colonies only. D-mannitol is fermented rapidly, lactose and sucrose more slowly. Some strains may ferment D-xylose. Catalase is produced. Indole is not produced. Ornithine is decarboxylated; arginine may be decarboxylated. Members are intestinal pathogens of humans and subhuman primates, causing bacillary dysentery. Humans are the primary reservoir. A long-term carrier state occurs in a small percentage of cases (Levine et al., 1973). Illness is typically milder than that caused by members of the other *Shigella* subgroups.

The mol% G + C of the DNA is: 51 (CsCl buoyant density).

Type strain: ATCC 29930, CIP 82.49, DSM 5570, NCTC 12984.

Other Organisms

S. boydii 13 strains were first described in 1952 by Ewing and Hucks (1952) under the provisional designation "serotype 425". Six years later, serotype 425 was added to the *Shigella* scheme as *S. boydii* 13 (Ewing et al., 1958). The initial description of the serotype included 10 strains: eight from cases and two from carriers isolated from persons from Italy, Belgian Congo, Egypt, and the United States. Except for differences in their ability to grow on ammonium salts glucose agar, the strains were biochemically indistinguishable from each other and most closely resembled shigellae of subgroup C. In 1973, Brenner et al. (1973a) reported evidence from DNA–DNA reassociation studies of *Shigella* species challenging the placement of *S. boydii* 13 strains in the genus *Shigella*. These investigators found that the *S. boydii* 13 strains they examined were highly interrelated but averaged only about 65% relatedness to other shigellae or members of the genus *Escherichia*. In a subsequent study, Brenner et al. (1982c) published findings from additional *S. boydii* 13 strains that extended and confirmed the earlier observation that *S. boydii* 13 strains, both anaerogenic and aerogenic varieties, are separable from other shigellae and *E. coli* based on DNA relatedness. The DNA–DNA reassociation evidence clearly demonstrates that *S. boydii* 13 strains represent a new, as yet unnamed, species. More recent findings from MLEE (Ochman et al., 1983; Pupo et al., 1997), nucleotide sequence analysis of housekeeping genes (Pupo et al., 2000), ribotyping (Rolland et al., 1998), and esterase electrophoretic polymorphisms (Goullet and Picard, 1987) are consistent with a distant relationship between *S. boydii* 13 strains and the other shigellae. At present, *S. boydii* 13 strains are primarily isolated by clinical laboratories and routinely keyed out as belonging to the genus *Shigella*. Because practical and logical factors are important in considering changes to existing systems of taxonomy and nomenclature (Brenner et al., 1973a), the renaming or reclassification of these bacteria is dependent on the development of methods that are useful for their identification in clinical laboratories.

Genus XXXVI. **Sodalis** *Dale and Maudlin 1999, 273[VP]*

COLIN DALE, SERAP AKSOY, SUSAN C. WELBURN, IAN MAUDLIN AND AHARON OREN

so.da' lis. L. masc. n. *sodalis* a companion.

Cells rod-shaped. Nonmotile. Gram negative. Endospore formation not observed. **Microaerophilic.** Chemoorganotrophic. Axenic growth occurs in media with enzymatically digested proteins serving as carbon and nitrogen sources. **Certain carbohydrates are used with the production of acids.** The optimum temperature for growth is 25°C, with little or no growth occurring at temperatures >30°C. Catalase and oxidase negative. **Found as secondary intracellular symbiotic bacteria in insects.**

The mol% G + C of the DNA is: 53.5.

Type species: **Sodalis glossinidius** Dale and Maudlin 1999, 273.

**Editorial Note:* This species is also known as subgroup B; formerly known as *"S. paradysenteriae"* Flexner.

**Editorial Note:* This species is also known as subgroup D.

Further descriptive information

The genus *Sodalis* consists of maternally transmitted intracellular symbiotic bacteria inhabiting the hemolymph and other organs of insects. Most intracellular symbiotic bacteria of insects are fastidious, and have thus far proved refractory to conventional culture techniques. *Sodalis glossinidius*, a multitissue secondary intracellular symbiont of the tsetse fly (*Glossina morsitans*), was the first true intracellular symbiont from insects to be cultivated *in vitro*. Cultivation was first achieved through the use of a mosquito (*Aedes albopictus*) feeder cell culture system. Subsequently, the organism was isolated in pure culture on a semidefined solid medium under microaerobic conditions. Therefore, *Sodalis* is the first example of the successful cultivation of an insect secondary intracellular symbiont on an agar-based medium. Phylogenetic characterization of *Sodalis* strains from different tsetse species revealed no differences based on their 16S rDNA sequences (Chen et al., 1999b). Other insects related to the tsetse fly carry bacterial symbionts with 16S rDNA sequences very similar to the 16S rDNA of *Sodalis*, but these symbionts are still awaiting isolation.

Sodalis glossinidius, the only species of the genus described thus far, is microaerophilic and can be cultivated only on solid media under a reduced-oxygen atmosphere. An atmosphere of 5% oxygen and 95% carbon dioxide is optimal for growth. Alternatively, aerotolerance-enhancing supplements such as catalase or fresh horse blood as a source of catalase activity can be added to the plates to enable growth in air.

The biochemical capacities of *Sodalis glossinidius* are rather restricted. Among the few carbohydrates used for growth the most efficient are *N*-acetyl-D-glucosamine and raffinose, which are metabolized with massive acid production. Glucose, glycol chitosan, mannitol, and sorbitol are metabolized to a lesser extent.

Sodalis glossinidius has a chromosome of about 2 Mb and about 134 kb plasmid DNA (Akman et al., 2001). Its chromosomal DNA is subject to extensive adenine and cytosine methylation. Hybridization of *Sodalis* DNA to *Escherichia coli* macroarrays revealed the presence of about 1800 orthologs, indicating that *Sodalis* has retained many of the capabilities of free-living bacteria. Based on this analysis, it appears that *Sodalis* has retained many genes involved in transcription, translation, regulation, nucleic acid, and amino acid biosynthetic pathways. However, it is possible that *Sodalis* might have lost genes in carbon compound catabolism, central intermediary metabolism, and fatty acid and phospholipid metabolism, which could account for the organism's restricted biochemical capacities.

To enter into insect cells, *Sodalis* uses a type III secretion system invasion gene (*invC*), very similar to virulence determinants previously identified in the genera *Salmonella* and *Shigella* (Dale et al., 2001).

Enrichment and Isolation Procedures

Sodalis glossinidius has been isolated from the hemolymph of *Glossina morsitans morsitans* in coculture with the mosquito *Aedes albopictus* cell line C6/36 (Welburn et al., 1987). Pure culture isolation was achieved through the use of solid-phase culture on serum-free Mitsuhashi-Maramorosch basal medium (Igarashi, 1978) with 1% Bacto-agar (Difco) under a microaerobic atmosphere (O_2/CO_2; 5:95). For growth under an air atmosphere, aerotolerance-enhancing supplements such as catalase or fresh horse blood cells (as source of catalase activity) should be added.

Maintenance Procedures

Sodalis glossinidius can be maintained by coculture in *Aedes albopictus* C6/36 cells at 25°C in liquid Mitsuhashi-Maramorosch medium (Igarashi, 1978) supplemented with 20% (v/v) heat-inactivated fetal calf serum by passaging the cells every 10 d (Welburn et al., 1987). For long-time storage bacterial cells are frozen in Mitsuhashi-Maramorosch medium supplemented with 20% (v/v) heat-inactivated fetal calf serum and 15% (v/v) glycerol.

Taxonomic Comments

Sodalis was classified in the family *Enterobacteriaceae* based on phenotypic tests and its 16S rDNA sequence. As a microaerophilic insect symbiont, it differs sufficiently from the other described genera within the *Enterobacteriaceae* to warrant classification as a separate genus. Different species and subspecies of tsetse flies harbor intracellular symbionts having almost identical 16S rDNA sequences (Beard et al., 1993; Aksoy et al., 1997). In addition, other insects, including flour and rice weevils and certain psyllids also harbor intracellular symbionts with closely related 16S rDNA sequences (Thao et al., 2000a).

Sodalis harbors type III secretion system invasion genes that are phylogenetically related to those found previously in *Salmonella enterica* and *Shigella flexneri*.

Further Reading

Akman, L., R.V.M. Rio, C.B. Beard and S. Aksoy. 2001. Genome size determination and coding capacity of *Sodalis glossinidius*, an enteric symbiont of tsetse flies, as revealed by hybridization to *Escherichia coli* gene arrays. J. Bacteriol. *183*: 4517–4525.

Dale, C. and I. Maudlin. 1999. *Sodalis* gen. nov. and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans* morsitans. Int. J. Syst. Bacteriol. *49*: 267–275.

Dale, C., S.A. Young, D.T. Haydon and S.C. Welburn. 2001. The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. Proc. Natl. Acad. Sci. U.S.A. *98*: 1883–1888.

List of species of the genus Sodalis

1. **Sodalis glossinidius** Dale and Maudlin 1999, 273.[VP]
glos.si.ni'di.us. N.L. adj. *glossinidius* of the genus *Glossinia*.

The characteristics are as described for the genus, with the following additional information. The cells are 1.0–1.5 × 2.0–12.0 μm. When grown intracellularly in *Aedes albopictus* cell culture, the organisms appear as pleomorphic rods. Temperature range for growth: 18–28°C. Colonies on Mitsuhashi-Maramorosch agar (Igarashi, 1978) are shiny, off-white, and concave, with entire edges.

Negative for DNase, gelatinase, urease, nitrate reductase, indole production, hippurate hydrolysis, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine decarboxylase, and starch hydrolysis. Produces α-galactosidase and β-*N*-acetylglucosaminidase. Does not produce α-fucosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, α-mannosidase, and β-xylosidase. Utilization of *N*-acetyl-D-glucosamine and raffinose accompanied by a high level of acid production. Utilizes glucose, glycol chitosan, mannitol, and sorbitol with accompanying weak acid production. No increase in growth is detected

when any of the following carbon sources are incorporated in the media: acetic acid, adonitol, δ-aminovaleric acid, L-arabinose, *n*-butanol, citric acid, dulcitol, ethanol, fructose, fumaric acid, galactose, glycerol, glycolic acid, histamine, *p*-hydroxybenzoic acid, α-ketoglutaric acid, lactose, D,L-malic acid, maltose, mannose, melibiose, methyl-α-D-glucopyranoside, *myo*-inositol, n-propanol, pyruvic acid, quinic acid, rhamnose, ribose, saccharic acid, salicin, sarcosine, sorbose, succinic acid, sucrose, starch, trehalose, or xylose.

Contains a number of large extrachromosomal DNA elements.

Found as secondary intracellular symbionts in the midgut, fat body, and hemolymph of the tsetse fly *Glossina morsitans morsitans*.

The mol% G + C of the DNA is: 53.5 (T_m).
Type strain: M1, NCIMB 13495.
GenBank accession number (16S rRNA): M99060.

Genus XXXVII. **Tatumella** *Hollis, Hickman and Fanning 1982, 267^{VP} (Effective publication: Hollis, Hickman, Fanning, Farmer, Weaver and Brenner 1981, 86) (Group EF-9 Hollis, Hickman, Fanning, Brenner and Weaver 1980)*

J.J. FARMER III

Ta.tum.el' la. M.L. dim. neut. *-ella* ending; M.L. fem. n. *Tatumella* named to honor Harvey Tatum, an American bacteriologist who made many contributions to our understanding of the classification and identification of fermentative and nonfermentative bacteria of medical importance.

Small rod-shaped cells 0.6–0.8 × 0.9–3 μm, conforming to the general definition of the family *Enterobacteriaceae*. Contains the enterobacterial common antigen. Gram negative. **Nonmotile at 36°C; over half the strains are motile by means of polar, subpolar, or lateral flagella when grown at 25°C.** Facultatively anaerobic. Catalase positive (weak and slow). Oxidase negative. Nonpigmented. **Stock cultures often die within a few weeks on laboratory media. Biochemically more active at 25°C than at 36°C.** Ferment, rather than oxidize, D-glucose; without the formation of visible gas. Reduce nitrate to nitrite. **Very inactive biochemically; positive tests only for Voges–Proskauer (Coblentz method), phenylalanine deaminase, and fermentation of sucrose, trehalose, and D-mannose. Negative for most tests**: indole production, methyl red, Voges–Proskauer (O'Meara method), citrate utilization (Simmons), H_2S production (TSI), urea hydrolysis, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, growth in the presence of cyanide (KCN test), malonate utilization, esculin hydrolysis, ONPG, gelatin hydrolysis (22°C), lipase (corn oil), DNase, gas production during fermentation, and the fermentation of lactose, D-mannitol, dulcitol, adonitol, *myo*-inositol, D-sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, cellobiose, α-methyl-D-glucoside, erythritol, D-arabitol, glycerol, and mucate. Have very large zones of inhibition around antibiotics; susceptible to colistin, nalidixic acid, sulfadiazine, gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, carbenicillin, ampicillin, and cephalothin (disk diffusion method on Mueller–Hinton agar). **Large zone of inhibition around a penicillin G (10 U) disk, in contrast to most other *Enterobacteriaceae*.**

Isolated from human clinical specimens, mainly from the **respiratory tract** where clinical significance is questionable. Also isolated from blood cultures suggesting clinical significance. Probably **a rare opportunistic pathogen or colonizer of humans**.

The mol% G + C of the DNA is: 53–54.

Type species: **Tatumella ptyseos** Hollis, Hickman and Fanning 1982, 267 (Effective publication: Hollis, Hickman, Fanning, Farmer, Weaver and Brenner 1981, 86.)

FURTHER DESCRIPTIVE INFORMATION

Literature Since *Tatumella* was described in 1981, there have been only a few reports in the literature. In addition to the genus and species names, "EF-9" should also be included as a search term in computerized literature searches, although it is rarely used today.

The *Tatumella* literature reports include reviews and taxonomic studies of new *Enterobacteriaceae* (Gilchrist, 1995; Aleksic and Bockemühl, 1999), and surveys or comparisons of the family *Enterobacteriaceae* for evolutionary relatedness based on 16S rRNA sequencing (Spröer et al., 1999), the enterobacterial common antigen (Ramia et al., 1982), catalase production (Chester and Moskowitz, 1987), metabolic pathways (Bouvet and Grimont, 1987a; Bouvet et al., 1989), polyamines (Hamana, 1996), and siderophores (Rabsch and Winkelmann, 1991).

Sources, strains, collections Strains of *Tatumella* have been isolated from a variety of human clinical specimens, but not from other sources. The original collection of Hollis et al. (1981) included isolates from body sites that are normally sterile such as blood (three isolates) and urine (one), but 38 were from throat, pharynx, tracheal aspirate, or sputum. One was from feces.

Clinical significance of human isolates The clinical significance and the ability of *T. ptyseos* to actually cause infections should be carefully evaluated in cases that yield this organism. Systematic study and good case reports are needed. Tan et al. (1989) reported a case of presumed sepsis in a neonate with jaundice who recovered uneventfully after treatment with antibiotics. This report and the three blood isolates described by Hollis et al. (1981) suggest that *T. ptyseos* should at least be considered as a rare opportunistic pathogen.

Isolation, identification, culture preservation, phenotypic characterization Strains of *Tatumella* are not difficult to grow, but may grow more slowly than typical *Enterobacteriaceae*. Stock cultures may die within a few weeks on agar or in semisolid stock culture media. This characteristic is very unusual, since most cultures of *Enterobacteriaceae* can be kept almost indefinitely in sealed tubes kept at room temperature. *Tatumella* cultures frozen in 5% rabbit blood and stored at −40°C to −60°C have remained viable after storage for up to 14 years. This latter method or storage in liquid nitrogen (or perhaps freeze-drying) should be suitable for long-term preservation. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for

biochemical testing, identification, computer programs, and antibiotic susceptibility. On MacConkey agar at 24 h, all strains grew and produced colorless (lactose negative) colonies.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* gives the results for *Tatumella* in 47 biochemical tests normally used for identification (Farmer, 1999). The most striking feature of *T. ptyseos* strains is their biochemical inactivity. They are also more active biochemically at 25°C than 36°C. The flagellation of *Tatumella* is also unusual. Strains are nonmotile at 36°C, but 66% are motile at 25°C. No flagella are produced on most cells, but those seen are polar, subpolar, or lateral rather than peritrichous. In contrast to most other *Enterobacteriaceae*, cultures of *T. ptyseos* have large zones of inhibition around 10 U penicillin G disks (range of 15–36 mm, mean of 24 mm, standard deviation of 4.6 mm). There are no genus- or species-specific tests or sequences for the identification of *Tatumella*. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the section on *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae*. Several computer programs greatly facilitate analyzing the results.

Taxonomic Comments

History The name *Tatumella* was coined by Hollis et al. (1981) for a group of organisms that had previously been known as "Group EF-9", a name coined and used by the Special Bacteriology Section at the Centers for Disease Control (Hollis et al., 1980). Group EF-9 had been known for many years, but its taxonomic position had not been studied.

Circumscription of *T. ptyseos* (Hollis et al., 1981) Twenty-seven "suspect" strains of Group EF-9 were studied by DNA–DNA hybridization, biochemical reactions, and antibiotic susceptibility. Twenty of the strains were very highly related to the type strain (89% or greater with ΔT_m values of 0–0.5) by DNA–DNA hybridization (60°C, hydroxyapatite method with ^{32}P). A group of six strains was less related (80–90% with ΔT_m values of 5.4–7.8), but was included in the species *T. ptyseos*. These strains may eventually be classified as one or more distinct biogroups. One strain (H34) was considerably less related (66% related at 60°C, which dropped to 44% at 75°C), and was not included in *T. ptyseos*. This probably represents a second species of *Tatumella*. By DNA hybridization, other taxa in the family *Enterobacteriaceae* were related by 7–38%, including 25–30% relatedness of *Escherichia*, the type genus of the family. Based on the DNA–DNA hybridization and phenotypic data, Group EF-9 was proposed as a new genus, *Tatumella*, with *T. ptyseos* as the only species (Hollis et al., 1981).

Phylogeny based on 16S rRNA sequencing A 16S rRNA tree that includes *T. ptyseos* can be found in Fig. BXII.γ.198 in the chapter on the genus *Budvicia* in this *Manual*. The 16S rRNA sequencing data agree with DNA–DNA hybridization and phenotypic data that *Tatumella* is a distinct genus of *Enterobacteriaceae*. This tree generally agrees with the tree published by Spröer et al. (1999), in that *Tatumella* is on a distinct branch. However, the two trees differ in the placement of the closest neighbors and other organisms (see also Fig.BXII.γ.189 in the chapter on the family *Enterobacteriaceae*).

Nomenclatural problem: the irregular author citation in the names *Tatumella* Hollis, Hickman and Fanning 1982, 267 [VP] and *Tatumella ptyseos* Hollis, Hickman and Fanning 1982, 267 [VP] The author citation for the names *Tatumella* and *Tatumella ptyseos* has traditionally been written as "Hollis, Hickman and Fanning 1982, 267 [VP]" rather than the expected citation of "Hollis, Hickman, Fanning, Farmer, Weaver and Brenner 1982, 267[VP]". The original paper (Hollis et al., 1981) had six authors rather than three, but on page 86 the authors stated: "We propose the following citations of the genus and species, which recognizes the greater contribution of three of the authors: *Tatumella* Hollis, Hickman and Fanning; *Tatumella ptyseos* Hollis, Hickman and Fanning."

This irregular author citation continued through correspondence with the editor of the *International Journal of Systematic Bacteriology* that requested validation of the genus and species name. In Validation List 8, the request for a "three-author citation" was granted and was without comment since only three authors, "Hollis, Hickman and Fanning", were listed under the column heading "Author(s)" for both names. This unusual citation appears to conflict with the rules of the Bacteriological Code (International Code of Nomenclature of Bacteria) for citing authors of new taxa. Although this is a small point, it is one that can lead to instability in nomenclature. The problem could be addressed by amending the Bacteriological Code, or through an Opinion of the Judicial Commission.

Further Reading

Hollis, D.G., F.W. Hickman, G.R. Fanning, J.J. Farmer, III, R.E. Weaver and D.J. Brenner. 1981. *Tatumella ptyseos* gen. nov., sp. nov., a member of the family *Enterobacteriaceae* found in clinical specimens. J. Clin. Microbiol. *14*: 79–88.

List of species of the genus Tatumella

1. **Tatumella ptyseos** Hollis, Hickman and Fanning 1982, 267[VP] (Effective publication: Hollis, Hickman, Fanning, Farmer, Weaver and Brenner 1981, 86.)
pty′ se.os. Gr. n. *ptyseos* a spitting (or less literally from sputum, the most common source of clinical isolates).

   The characteristics are as given for the genus and the results for 47 biochemical tests are given in Table BXII.γ.193 of the chapter on the family *Enterobacteriaceae*. Isolated from human clinical specimens, primarily from the respiratory tract; should probably be considered a rare opportunistic pathogen.

   The mol% G + C of the DNA is: 53–54 (Bd).

   Type strain: H36, ATCC 33301, CDC D6168, CDC 9591-78, DSM 5000.

   GenBank accession number (16S rRNA): AJ233437.

   Additional Remarks: Hollis et al. (1981) include 25 other strains in the species; however, six of these were less related to the type strain that the others (see previous discussion). One additional strain (very highly related to the type strain) was also deposited in the American Type Culture Collection (ATCC 33302 [H3 9558-78 = A7744]), from human sputum, Connecticut, USA.

Genus XXXVIII. **Trabulsiella** *McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1992, 327^{VP} (Effective publication: McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1991, 1482) (Enteric Group 90 Murlin, Brenner, Steigerwalt and Farmer 1988)*

JOHN A. LINDQUIST AND J.J. FARMER III

Tra.bul.si.el' la. M.L. dim. ending *-ella;* M.L. fem. n. *Trabulsiella* named to honor L.R. Trabulsi, a Brazilian bacteriologist who did many important studies on the enteric pathogens of the family *Enterobacteriaceae*.

Rod-shaped cells, conforming to the general definition of the family *Enterobacteriaceae*. Gram negative. Motile. Facultatively anaerobic. Catalase positive. Oxidase negative. Ferment, rather than oxidize, D-glucose and other carbohydrates and produce visible gas during fermentation. Reduce nitrate to nitrite. **Positive for methyl red, citrate utilization (Simmons), H_2S production (TSI), lysine decarboxylase, ornithine decarboxylase, motility at 36°C, growth in the presence of cyanide (KCN test), esculin hydrolysis, ONPG, tyrosine hydrolysis, and the fermentation of D-mannitol, salicin, D-sorbitol, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, esculin, mucate, D-mannose, and D-galactose. Arginine dihydrolase is positive, but delayed.** Negative for Voges–Proskauer, urea hydrolysis, phenylalanine deaminase, malonate utilization, gelatin hydrolysis (22°C), lipase (corn oil), DNase, and the fermentation of sucrose, dulcitol, adonitol, *myo*-inositol, raffinose, α-methyl-D-glucoside, erythritol, melibiose, and D-arabitol. **Biochemically similar to, and can be confused with, *Salmonella* in routine screening tests. Do not completely type in *Salmonella* O or H antisera, and are negative in the *Salmonella*-specific test: lysis by bacteriophage O1, MUCAP (4-methylumbelliferyl caprylate), and a commercial gene probe (Gene-Trak Systems).** Susceptible to colistin, nalidixic acid, gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, and carbenicillin; **resistant to penicillin, ampicillin, and cephalothin**; variable resistance to sulfadiazine and carbenicillin (disk diffusion method on Mueller–Hinton agar).

Ecological niches are not known, but have been isolated from environmental samples and human feces. However, there is **no evidence that *Trabulsiella* causes diarrhea or intestinal infections**. Apparently a **very rarely isolated genus of *Enterobacteriaceae***.

The mol% G + C of the DNA is: not determined.

Type species: **Trabulsiella guamensis** McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1992, 327 (Effective publication: McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1991, 1483.)

FURTHER DESCRIPTIVE INFORMATION

Literature Since *Trabulsiella* was described in 1991, there have been only a few reports in the literature. In addition to the genus and species names, "Enteric Group 90" should also be included as a search term in computerized literature searches, although it is rarely used today.

Sources Six strains of *Trabulsiella* were isolated from vacuum cleaner dust on the island of Guam, three were from human stool specimens (one from New York, USA, and two from Germany), one was from wheat flour from Oregon, USA, and two were from environmental material (not further specified) in Malaysia. Many of these isolates were originally isolated and studied as "suspect *Salmonella* cultures".

Isolation, identification, culture preservation, phenotypic characterization Strains of *Trabulsiella* are not difficult to grow, and are typical *Enterobacteriaceae* in most respects. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility. Strains of *Trabulsiella* will often appear as typical *Salmonella* strains on some enteric plating and screening media; however, they do not type completely in *Salmonella* O and H antisera.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the section on the family *Enterobacteriaceae* gives the results for *Trabulsiella* in 47 biochemical tests normally used for identification (Farmer, 1999). There are no genus- or species-specific tests or sequences for the identification of *Trabulsiella*. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the chapter on the family *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 of that chapter. Several computer programs greatly facilitate analyzing the results. *Trabulsiella* strains are phenotypically most like *Salmonella* subgroups 4 and 5, which are rarely isolated from human clinical specimens.

TAXONOMIC COMMENTS

In 1985 the vernacular name Enteric Group 90 was coined for a group of strains that had been studied at the *Salmonella* Laboratory at the Centers for Disease Control and Prevention. From 1985 to 1988 eight strains were characterized that were phenotypically distinct from all of the described organisms in the family *Enterobacteriaceae*. They had been submitted as "biochemically *Salmonella*, but will not serotype". Since the strains were biochemically very close to *Salmonella*, particularly to *Salmonella* DNA hybridization groups 4 and 5, they were originally suspected as being a new DNA hybridization group in the genus *Salmonella*. However, by DNA–DNA hybridization the strains were very highly related to each other, but were more distantly related to other organisms in the family *Enterobacteriaceae*. Strain ATCC 49490 (CDC 370-85) that eventually became the type strain of *Trabulsiella guamensis* was 41% related by DNA–DNA hybridization to *Salmonella* serotype Typhimurium and to a strain of *Salmonella* DNA hybridization group 3b. Less relatedness was found to *Kluyvera ascorbata* (39%), *Shigella flexneri* (38%), *Klebsiella terrigena* (38%), and strain 6003-71 of the *Enterobacter agglomerans* complex (38%). Other *Enterobacteriaceae* were 6–37% related. Because strains of Enteric Group 90 were distinct by both DNA–DNA hybridization and phenotype, a new genus *Trabulsiella* with a single species *Trabulsiella guamensis* was proposed (McWhorter et al., 1991). *Trabulsiella* and *Trabulsiella guamensis* gained standing in nomenclature in 1992 when they appeared on Validation List 41 (McWhorter et al., 1992).

Further Reading

McWhorter, A.C., R.L. Haddock, F.A. Nocon, A.G. Steigerwalt, D.J. Brenner, S. Aleksic, J. Bockemuehl and J.J. Farmer, III. 1991. *Trabulsiella guamensis*, a new genus new species of the family *Enterobacteriaceae* that resembles *Salmonella* subgroups 4 and 5. J. Clin. Microbiol. *29*: 1480–1485.

List of species of the genus Trabulsiella

1. **Trabulsiella guamensis** McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1992, 327[VP] (Effective publication: McWhorter, Haddock, Nocon, Steigerwalt, Brenner, Aleksic, Bockemühl and Farmer 1991, 1483.)
guam.en'sis. M.L. adj. *guamensis* pertaining to Guam, the largest island of the Micronesian group of the Pacific Ocean, where the first strains were isolated.

The characteristics are as given for the genus. Isolated from environmental samples, food, and human feces. However, there is no evidence that *Trabulsiella guamensis* can cause diarrhea or intestinal infections. Apparently it is a very rarely isolated species of *Enterobacteriaceae*, whose importance in microbiology may be more as a nuisance, because of its biochemical similarity to *Salmonella* and possible misidentification as *Salmonella*. The type strain was isolated from vacuum cleaner contents on the island of Guam obtained during a survey to isolate *Salmonella* from environmental reservoirs, and to look at the role of reptile feces as a source of *Salmonella* infections. Vacuum cleaner contents were a convenient sample of "indoor dirt".

In the addendum section of their paper, McWhorter et al. (1991) described two different biochemical patterns given by *T. guamensis* strains, but they did not name them as biogroups. The eight strains from the United States and Guam were negative for indole production, gelatin hydrolysis (film method, 36°C), and esculin hydrolysis (within 2 d), but the four strains from Germany and Malaysia were positive. Strain 2421-87 (feces, Germany) was the only one of the original eight strains studied that had this latter pattern, but it was 98% related to the type strain by DNA–DNA hybridization at 60°C and the value did not drop when the temperature was raised to 75°C. Thus, the available data indicate that the two groups of strains should be considered as biogroups of *T. guamensis*.

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 49490, CDC 0370-85.

Additional Remarks: The American Type Culture Collection includes four other strains of *Trabulsiella guamensis* including strain ATCC 49493 (CDC 2421-87, from human feces, Germany) that is from the indole-positive biogroup described above.

The biochemical and geographic differences of the two groups of strains may reflect evolutionary divergence that should be investigated with methods other than DNA–DNA hybridization that measure relatedness. J.J. Farmer (unpublished data) recently found a group of three strains in the collection of the Enteric Reference Laboratory, CDC that were very similar to *Trabulsiella*, but were H_2S negative. These could represent a second species of *Trabulsiella*.

Genus XXXIX. **Wigglesworthia** *Aksoy 1995b, 849[VP]*

Serap Aksoy

Wigg.les.worth'i.a. M.L. fem. n. *Wigglesworthia* named after the parasitologist W.B. Wigglesworth.

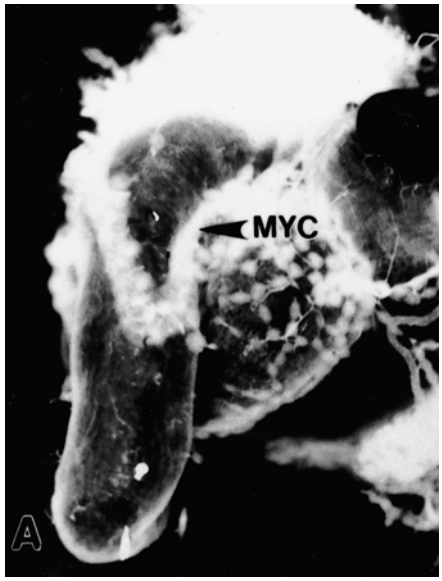
Obligate intracellular, bacteriome-tissue associated, primary endosymbionts of tsetse flies. In the adult fly, the bacteria lie free in the cytoplasm of differentiated epithelial cells, bacteriocytes, which form the U-shaped bacteriome organ (previously referred to as mycetome) in the anterior midgut (Figs. BXII.γ.210A, B). The endosymbionts are rod shaped (2–4 × 8–10 μm) with a typical Gram-negative cell wall and inner membrane structure, lack flagella (Fig. BXII.γ.210C). The cells appear to divide by binary fission. The bacteria are obligate intracellular organisms, as their experimental elimination from tsetse by procaryotic-specific antibiotic treatment, by lysozyme, or with symbiont-specific antibodies provided in the blood meal results in retarded growth of the insect and a decrease in egg production, causing loss of the ability of these aposymbiotic hosts to reproduce (Hill et al., 1973; Nogge, 1976, 1978, 1980). In addition to *Wigglesworthia*, tsetse harbors a secondary (S)-symbiont in midgut tissue. The specific function(s) of *Wigglesworthia* and S-symbionts are not known; however, since the ability to reproduce can be partially restored when the aposymbiotic tsetse receive a blood meal that is supplemented with B-complex vitamins (thiamine, pantothenic acid, pyridoxine, folic acid, and biotin), it is suggested that the gut-tissue associated endosymbionts may play a role in metabolism that involves these compounds (Nogge, 1981). The recently sequenced genome of *Wigglesworthia* shows the presence of various biosynthetic pathways for vitamin metabolites (Akman et al., 2002). The 16S rDNA-based phylogeny of *Wigglesworthia* characterized from eight different tsetse species displays concordance with their insect host species, indicating an ancient association for this bacterium with an ancestral tsetse that has subsequently radiated along with the host insect species (Chen et al., 1999b). The bacterium is not present in the ovaries, but may be transmitted to the intrauterine larvae of tsetse via its milk-gland secretions.

Type species: **Wigglesworthia glossinidia** Aksoy 1995b, 849.

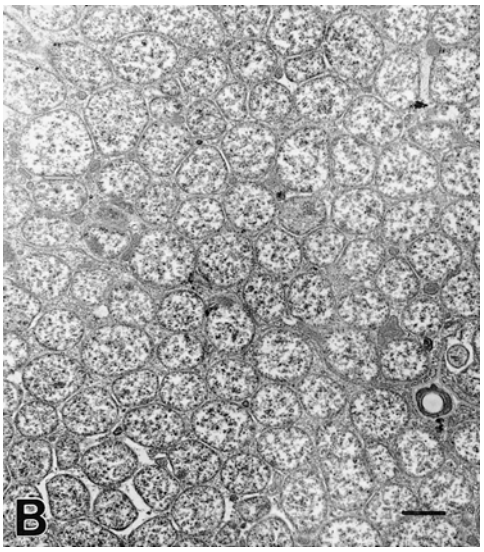
Further descriptive information

Various insects that rely on a single food source (blood or phloem) have established symbiotic associations with microorganisms in order to supplement their limited diets. Stuhlman found bacteria in the intestines of tsetse (Stuhlmann, 1907) and Roubaud described a mycetome (bacteriome)-like organ in midgut (Rouboud, 1919) that was confirmed by Wigglesworth and Buxton (Wigglesworth, 1929; Buxton, 1955). In ultrastructural studies, Reinhardt et al. (1972) described the large bacteroid found in tsetse mycetomes, and Pinnock and Hess (1974) distinguished these organisms from the smaller S-symbionts found in midgut tissue.

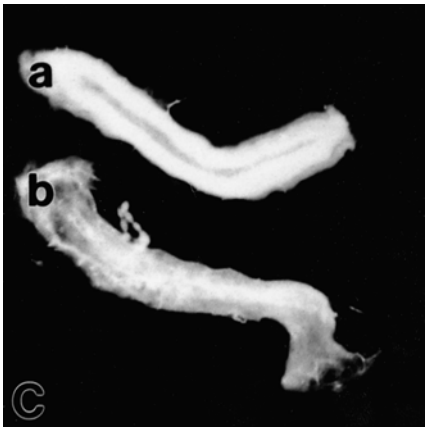
The well-defined bacteriome structure in tsetse guts can be



A



B



C

FIGURE BXII.γ.210. Bacteriome structure and *Wigglesworthia* in bacteriocytes. *A*, The *arrow* points at the U-shaped bacteriome (previously called mycetome, MYC) structure in the anterior midgut region of *G. m. morsitans*. (Reproduced with permission from S. Aksoy et al., Insect Molecular Biology, *4:* 15–22, 1995, ©Blackwell Science Ltd., Oxford.) *B*, Electron micrograph of *Wigglesworthia* showing the packed endosymbionts in the bacteriocytes of *G. morsitans*. The endosymbionts are surrounded by a double-membrane (× 5000; bar = 4 μm). *C*, Dissected bacteriomes: (*a*) from a female maintained on a regular blood meal, and (*b*) from female that has received an antibiotic containing blood meal for one week. The bacteriome structure in the presence of antibiotics has disintegrated. (Reproduced with permission from S. Aksoy et al., Insect Molecular Biology, *4:* 15–22, 1995, ©Blackwell Science Ltd., Oxford.)

dissected and transiently maintained in tissue culture (Wink, 1979). Gene expression studies *in vivo* and *in vitro* have shown one of the major gene products of *Wigglesworthia* to be a molecular-chaperonin, the *groEL* homolog of *Escherichia coli* (Aksoy, 1995a). The overexpression of stress-related proteins has also been reported for the bacteriome endosymbionts of aphids (Kakeda and Ishikawa, 1991) and may be due to the intracellular nature of these microorganisms. PCR-based experiments show that *Wigglesworthia* is not present in ovary tissue (O'Neill et al., 1993), thus ruling out a transovarial transmission route; however, previous microscopy studies have reported the presence of microorganisms with similar morphology in milk-gland tissue of tsetse (Ma and Denlinger, 1974). Thus, milk-gland secretions may provide a route for the maternal transmission of *Wigglesworthia* to the intrauterine larvae of tsetse.

The S-symbionts of tsetse harbored in midgut-tissue and *Wolbachia* harbored in ovary tissue are morphologically different from *Wigglesworthia*. They are short rods (1–2 μm in length), and inside host cells they are surrounded by host membranes. There is a clear lytic zone around the cells reminiscent of true *Rickettsiaceae* to which the genus *Wolbachia* is related. Since phylogenetic analysis of tsetse S-symbionts has shown them to be enteric microorganisms, it is not clear what this lytic zone signifies (Reinhardt et al., 1972). The organization and copy number of the 16S rDNA gene in *Wigglesworthia* is different from *Buchnera* (the phylogenetically closely related P-endosymbionts of aphids), tsetse S-symbionts, and *E. coli*. The free-living bacterium *E. coli* and tsetse S-symbionts have multiple copies of rDNA operons encoding the 16S and 23S gene products. *Wigglesworthia* was shown to have a single operon encoding its 16S rDNA by Southern blot analysis (Aksoy, 1995a). The genome sequence analysis now indicates that there are two copies of the rDNA operon in *Wigglesworthia*, unlike the single operon found in *Buchnera* (Akman et al., 2002). In *Buchnera*, the 16S and 23S rDNA genes are not genetically linked, while in *Wigglesworthia* they are found to be transcriptionally linked (Aksoy, 1995a; Akman et al., 2002).

Taxonomic Comments

While it has not been possible to maintain the bacteriome-tissue symbionts *in vitro*, recent PCR-based amplification techniques and the use of nucleic acid sequences in phylogenetic recon-

structions have provided additional insight into the relationships among these intracellular bacteria. Their characterization from different species of aphids, whiteflies, carpenter ants, and tsetses have shown that they each form unique lineages within the *Gammaproteobacteria*, while the organisms from mealybugs are in the *Betaproteobacteria* and those associated with cockroaches are members of flavibacteria. Their phylogeny in each case has been found to parallel the phylogeny of their host insect species, indicating the ancient and obligatory nature of these symbiotic relationships (Baumann et al., 1998). The phylogeny of *Wigglesworthia* was analyzed from different flies representing the four species groups of genus *Glossina* (*morsitans*, *palpalis*, *machadomyia*, and *fusca*). For this analysis, the 16S rDNA sequence that is present as a single-copy gene was used (Aksoy et al., 1995; Chen et al., 1999b). The phylogenetic relationships of the tsetse host species were also analyzed by using an insect gene, the internal transcribed spacer-2 region of rDNA (ITS-2). These sequences were then used to generate phylogenetic alignments by maximum parsimony and two representative trees are shown in Fig. BXII.γ.211: for *Wigglesworthia* (Fig. BXII.γ.211b) and for the tsetse host species (Fig. BXII.γ.211a). The alignment of *Wigglesworthia* included the tsetse midgut S-symbiont clade and the S-symbionts of the weevil, *Sitophylus zeamais*; *Escherichia coli* was chosen as the out group. All trees generated could distinguish the tsetse symbionts, *Wigglesworthia*, and midgut S-symbionts, as distinct lineages within the *Gammaproteobacteria*. While within the S-symbiont clade no differences were observed in their 16S rDNA sequence, *Wigglesworthia* analyzed from the fusca group (*G. brevipalpis*) was found

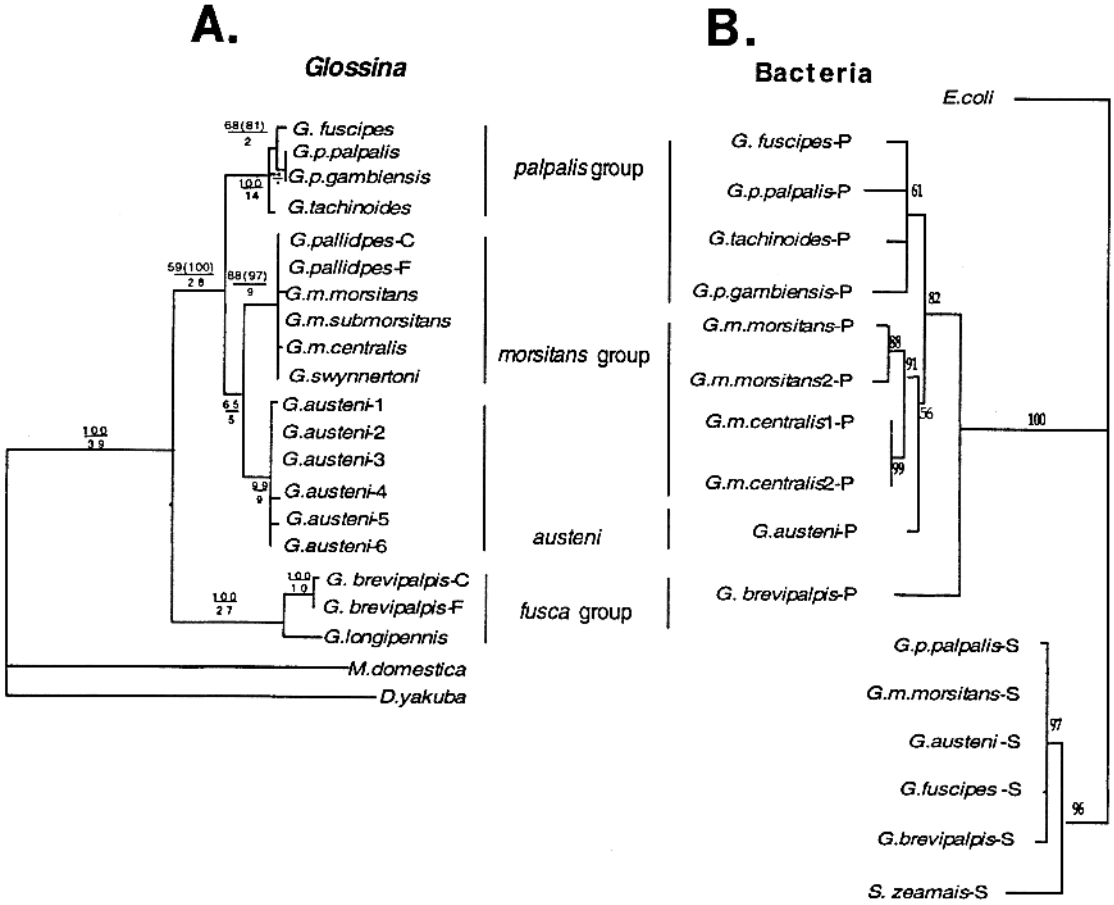


FIGURE BXII.γ.211. The phylogenetic placement of *Glossina* species and their symbionts. (Reproduced with permission from X. Chen et al., Journal of Molecular Evolution, *48:* 49–58, 1999, ©Springer-Verlag.) *A*, The phylogenetic tree constructed by maximum parsimony using the ITS-2 sequence. Tree length = 292, CI = 0.897. The bootstrap confidence values (300 replications) are presented at the nodes; the values in parentheses are bootstrap values where gaps have been included as weighted characters; numbers below denote branch length values. *D. yakuba* and *M. domestica* sequences are used as out groups. The grouping of the *palpalis*, *morsitans*, *austeni*, and *fusca* species are indicated. The origination of the *G. austeni* flies are as follows: *1*, colony maintained in Seibersdorf tsetse laboratories originating from Tanzania; *2*, field collected from Zanzibar; *3*, field collected from Shimba Hills (Kenya); *4*, colony established from Shimba Hills (Kenya); *5* and *6*, field collected from Hell's Gate Park in South Africa. *C* denotes flies analyzed from colonies. *F* denotes field isolates. *G. pallidipes*-C is from the colony at International Livestock Research Institute (ILRI) originating from Shimba Hills area of Kenya. *G. pallidipes*-F was a field collected fly from Nguruman region of Kenya. *G. brevipalpis*-C is from the colony at ILRI established from Kenya. *G. brevipalpis*-F is a field sample from South Africa. *B*, Phylogenetic analysis of *Wigglesworthia* and midgut S-symbionts based on their 16S rDNA sequence analysis. P denotes primary symbiont *Wigglesworthia*, and S denotes the secondary symbionts analyzed from the corresponding species of *Glossina*. *E. coli* was used as the out group. The analysis included 893 sites, of which 205 were variable and 121 were informative. One representative tree is shown (tree length 216, CI = 0.843, bootstrap values for 500 replications are shown at the nodes). *G. m. centralis*-1 was obtained from the colony maintained at ILRI in Kenya, while *G. m. centralis*-2 is from the colony maintained at Alberta and has originated from Zambia. The GenBank accession numbers for the 16S rDNA loci of *Wigglesworthia* from *G. p. gambiensis*, *G. m. morsitans*-2, *G. m. centralis*-1, *G. m. centralis*-2, *G. austeni*, and *G. fuscipes* are AF022875–AF022880, respectively.

to occupy the deepest branch. The *Wigglesworthia* sequences analyzed from the *morsitans* and *palpalis* group species formed sister taxa. The placement of *Wigglesworthia* from *G. austeni* (*machadomyia*), however, was less certain, since equally robust trees could be generated that differed only in the placement of *G. austeni* with respect to the other two subgenera. The low bootstrap-analysis confidence value (56) at the node where *G. austeni* symbiont forms a sister group with the *morsitans* species demonstrates this uncertainty. The ITS-2 sequence-based alignment (Fig. BXII.γ.211a) displayed similar phylogenetic relationships among the tsetse host species; the fusca flies were found to be the most divergent, while the *palpalis*, *morsitans*, and *machadomyia* subgenera were of more recent origin, and again the evolutionary relatedness of *machadomyia* with respect to the *palpalis* and *morsitans* subgenera could not be resolved with high confidence. The comparative analysis of the two phylogenies indicates their strong similarity, arguing for concordance of *Wigglesworthia* with their host tsetse species.

List of species of the genus Wigglesworthia

1. **Wigglesworthia glossinidia** Aksoy 1995b, 849[VP]
glos.si.nid′i.a. M.L. fem. adj. *glossinidia* referring to *Glossina*, the genus of tsetse flies.

The characteristics are as described for the genus and as depicted in Fig. BXII.γ.210.

The mol% G + C of the DNA is: not known.

Type strain: endosymbiont of Glossina morsitans morsitans.

GenBank accession number (16S rRNA): L37339.

Genus XL. **Xenorhabdus** *Thomas and Poinar 1979, 354[AL] emend. Thomas and Poinar 1983, 878*

Raymond J. Akhurst and Noël E. Boemare

Xe.no.rhab′dus. Gr. n. *xenos* unwanted guest (less literally, pathogen); Gr. fem. n. *Rhabdus* rod; M.L. masc. n. *Xenorhabdus* pathogenic rod-shaped bacterium.

Asporogenous, rod-shaped cells 0.3–2 × 2–10 μm and occasionally with filaments 15–50 μm in length. Spheroplasts, averaging 2.6 μm in diameter, appear in the last third of exponential growth. **Proteinaceous crystalline inclusions** develop in a large proportion of cells in stationary phase cultures. Gram negative. Motile by means of peritrichous flagella. Swarming may occur on 0.6–1.2% agar. Facultatively anaerobic, with both respiratory and fermentative types of metabolism. **Optimum temperature usually ~28°C or less**; a few strains grow at 40°C. Acid (no gas) production from glucose; ferment some other carbohydrates but poorly. **No catalase activity; nitrate is not reduced to nitrite.** Negative for most tests used to differentiate *Enterobacteriaceae*. Lipase detected with Tween 20 and egg yolk agar; most strains lipolytic on Tween 40, 60, 80, and/or 85. Positive for deoxyribonuclease and protease. **Phase shift occurs to varying degrees in stationary phase cultures**, giving rise to phase II, which lacks dye adsorption, antibiotic production, protein inclusions, and some other characteristics of the phase I isolated from the natural environment. **Only known from the intestinal lumen of entomopathogenic nematodes of the family** *Steinernematidae* and insects infected by these nematodes.

The mol% G + C of the DNA is: 43–50 (Bd).

Type species: **Xenorhabdus nematophila** (Poinar and Thomas 1965) Thomas and Poinar 1979, 355 (*Achromobacter nematophilus* Poinar and Thomas 1965, 249.)

Further descriptive information

Analyses of 16S rDNA sequences show that *Xenorhabdus* is most closely related to *Photorhabdus*, a genus composed largely of bacteria symbiotically associated with entomopathogenic nematodes of the family *Heterorhabditidae*. The next nearest phylogenetic neighbors are *Proteus vulgaris* and *Arsenophonus nasoniae* (Suzuki et al., 1996; Brunel et al., 1997; Liu et al. 1997a; Szállás et al., 1997).

Xenorhabdus species are insect pathogenic bacteria that occur naturally in the intestinal vesicle of nonfeeding infective stage entomopathogenic nematodes of the family *Steinernematidae* (Bovien, 1937; Poinar and Leutenegger, 1968; Bird and Akhurst, 1983). After invading an insect host, the nematode commences development, releasing *Xenorhabdus* into the nutrient-rich hemolymph. The bacteria proliferate, killing the insect host and producing suitable nutrient conditions for growth and reproduction of the nematodes as well as an array of antibiotics and bacteriocins to minimize competition. As the nutrient source becomes depleted, the immature nematodes develop into the infective stage that will transport *Xenorhabdus* to a new nutrient source. The association between each *Xenorhabdus* species and nematode is specific, with each nematode only naturally associated with a single *Xenorhabdus* species. However, *X. bovienii*, *X. poinarii*, and *X. beddingii* are each associated with more than one *Steinernema* species (Akhurst, 1983a, 1986b; Fischer-Le Saux et al., 1998).

A highly significant feature of bacteria of the genus *Xenorhabdus* is a phase variation that occurs in stationary phase (Akhurst, 1980). Only phase I *Xenorhabdus* have been detected in nature, but under *in vitro* conditions a variable proportion undergoes a profound change affecting colony and cell morphologies, motility, endo- and exo-enzymes, including respiratory enzymes, and secondary metabolites (Akhurst, 1980; Boemare and Akhurst, 1988; Smigielski et al., 1994; Givaudan et al. 1995; Fodor et al. 1997; Table BXII.γ.277). The timing and extent of phase change is largely unpredictable, but the rate of change from phase I to phase II is generally greater than the reverse. Phase I *Xenorhabdus* provide and protect essential nutrients for the nematodes by killing and metabolizing the insect host and producing a range of antimicrobial agents. Although phase II variants may also kill the insect host, they are less effective in providing growth conditions for the nematodes (Akhurst, 1980, 1982a), and have never been found associated with naturally occurring nematodes; the role of phase II is uncertain.

Xenorhabdus cells are rod-shaped cells that are highly variable in size, ranging from 0.3 × 2 μm to 2 × 10 μm with occasional

filaments 15–50 μm in length. Varying proportions of the cells occur as spheroplasts (diameter ~2.6 μm) in stationary phase cultures. Proteinaceous inclusion bodies, commonly two morphs, are evident in many of the rod-shaped phase I cells in stationary phase (Fig. BXII.γ.212). The glycocalyx surrounding *Xenorhabdus* cells is irregular in thickness, with a mean depth of 142 nm in phase I *X. nematophila** and 49 nm in phase II (Brehélin et al.,

**Editorial Note: Xenorhabdus nematophila* was originally named *Xenorhabdus nematophilus*; the name was corrected by Euzéby and Boemare (2000).

TABLE BXII.γ.277. Differential characteristics for phase I and II of *Xenorhabdus* and *Photorhabdus* species[a]

	1. *X. nematophila*		2. *X. beddingii*		3. *X. bovienii*		4. *X. japonica*		5. *X. poinarii*	
Characteristic[b]	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Colonial properties:										
Morphology[c]	g	t	g	t	g	t	g	nr	g	t
Dye adsorption	+	−	+	−	+	−	+	−	+	−
Pigmentation[d]	ow	ow	lb	ow	y	ow	yb	nr	b	ow
Ultrastrutural elements and cytological properties:										
Protoplasmic inclusions	+	−	+	−	+	−	nr	nr	+	−
Enzymatic activities:										
Antibiotics	+	+w	+	−	+	−	+	−	d	d
Lecithinase (egg yolk agar)	d	−	+	−	[+]w	−	+	−	−	−
Phospholipase (lecithin agar)	+	dw	+	−	+	−	nr	nr	[−]	−
Lipolysis (Tween 80)	[−]	+	+	+	+	+	−	−	+	+
Simmons citrate	+	+	+	+	−	+	nr	nr	d	+
Phenylalanine deaminase	−	d	−	−	−	−	+	−	−	−
Gelatin hydrolysis	+	d	+	+	+	+	+	−	+	+

[a]All tests were done at 28° ± 1°C for *Xenorhabdus* and *Photorhabdus*. Data compiled from the following references: Akhurst (1980, 1982a, 1986a), Boemare and Akhurst (1988); Brehélin et al. (1993); Nishimura et al. (1994).

[b]Symbols: +, 90–100% of strains are positive; [+], 76–89% are positive; d, 26–75% are positive; [−], 11–25% are positive; −, 0–10% are positive; w, weak reaction.

[c]g, granular; t, translucent; nr, not reported.

[d]ow, off-white; y, yellow; b, brown; lb, light brown; yb, yellowish brown.

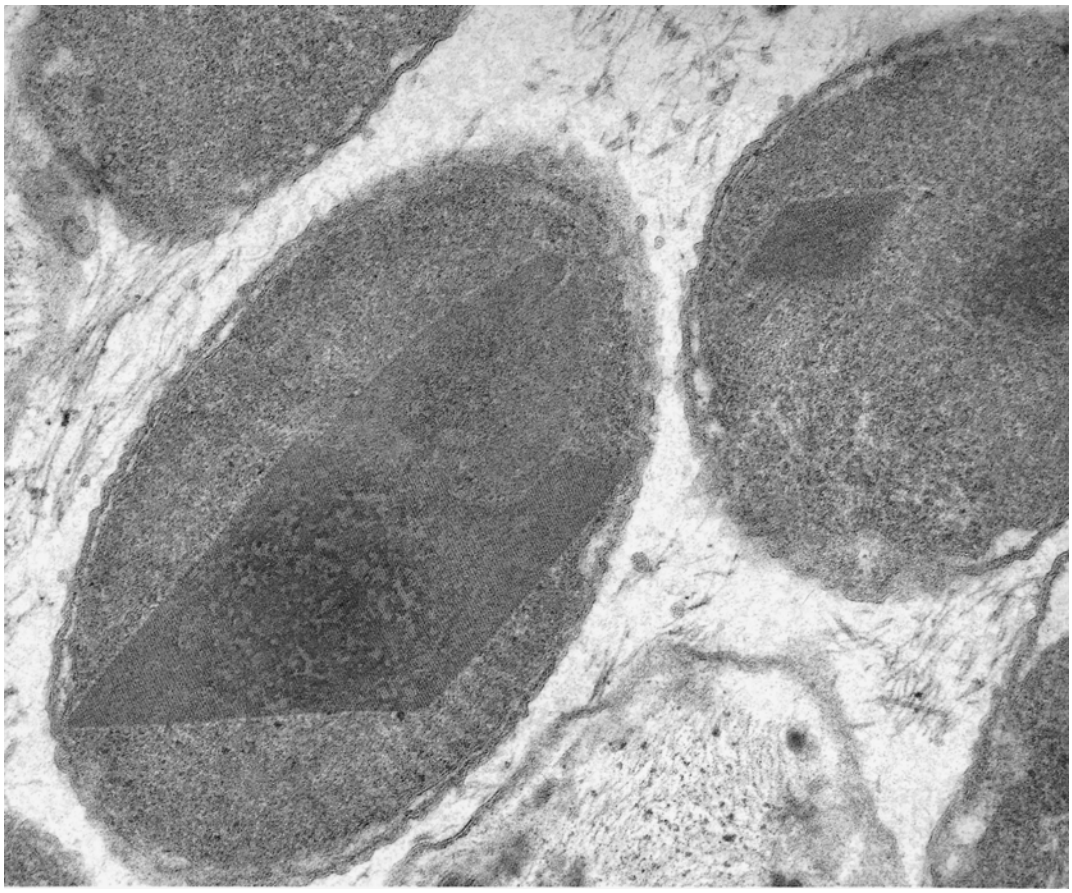


FIGURE BXII.γ.212. Protein inclusions in *X. nematophila*, showing a rhomboidal crystalline inclusion and an ovoid inclusion body.

1993). Phase I, but not phase II, cells have a peritrichous array of fimbriae with diameter of 6.4 nm, morphologically similar to the type I fimbriae of *E. coli* (Binnington and Brooks, 1993; Moureaux et al., 1995).

Phase I *Xenorhabdus* such as those isolated from the nematode associates or insects infected by the nematodes form convex, circular colonies with slightly irregular margins and a diameter of 1.5–2 mm after 4 d at 28°C; they also have a slightly granular appearance and, in some species, are pigmented (yellow, brown). These colonies adsorb dyes, such as bromothymol blue and neutral red, taking on intense coloration. Phase II variants generally form similar colonies but are flatter and wider (diameter 2.5–3.5 mm after 4 d at 28°C) and with lesser pigmentation; these colonies adsorb dyes only very weakly and no coloration is evident in 4-d colonies.

The major cellular fatty acids of *Xenorhabdus* are $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, and $C_{17:0\ cyc}$, and the respiratory quinone system is ubiquinone-8 (Suzuki et al., 1990).

Xenorhabdus species are easily grown *in vitro* on a range of complex liquid and solid media and in minimal media supplemented with nicotinic acid, *p*-aminobenzoic acid, serine, tyrosine, and/or proline (Grimont et al., 1984b); nutrient agar is suitable for all strains. They are mesophilic; most grow between 15 and 30°C but strains growing at 4°C or at 40°C have been isolated.

On the basis of 16S rDNA sequence data, *Xenorhabdus* can be distinguished from its nearest phylogenetic neighbor, *Photorhabdus* (Fig. BXII.γ.213), by the sequence TTCG at positions 208–211 (*E. coli* numbering) of the 16S rDNA where *Photorhabdus* has a longer version (TGAAAG) (Szállás et al., 1997). DNA–DNA hybridization demonstrated the existence of a number of undescribed species in the genus (Fig. BXII.γ.214). Data have been recorded for some of the strains that would be assigned to new *Xenorhabdus* species (Boemare and Akhurst, 1988) but too few to warrant a decision on their taxonomic status; there are no 16S rDNA data for any of these strains.

Conjugation of plasmids from *E. coli* has been applied successfully for *X. nematophila* and *X. bovienii* (Smigielski and Akhurst, 1992; Francis et al. 1993; Forst and Nealson, 1996). In contrast, transformation of *Xenorhabdus* has not been generally successful. Although Xu et al. (1989) reported the transformation of the type strain of *X. nematophila* with a broad host-range vector, they and other workers have been unsuccessful with other strains of *X. nematophila* and other *Xenorhabdus* spp. The increase in transformation efficiency after the vector had been passed through *X. nematophila* suggested the presence of a restriction modification system. This suggestion was further supported by the demonstration that *Xenorhabdus* DNA is not easily digested by several common restriction enzymes (Akhurst et al., 1992). Endonuclease activity has subsequently been detected in a range of *Xenorhabdus* spp. (Akhurst et al., 1992).

Hypovirulent and avirulent mutants have been produced in *X. nematophila* by chemical and transposon mutagenesis (Xu et al., 1991; Dunphy, 1994). Francis et al. (1993) developed a transposon mutagenesis system for *X. bovienii* by constitutively expressing *lamB* on the surface of the bacterium, allowing them to be infected with lambda particles carrying the Tn10 transposon. This process produced various dye-binding, lipase, protease, hemolytic, and DNase mutants.

Couche et al. (1987) demonstrated the presence of one or two small plasmids (3.6–12 kb) in some, but not all, *X. nematophila* and *X. bovienii* strains. The plasmid profiles did not differ between phases I and II. Smigielski and Akhurst (1994) reported two megaplasmids (71.8 and 118.5 kb) as well as two additional plasmids (6.5 and 17 kb) in the A24 strain of *X. nematophila*. They also demonstrated that all strains of *X. nematophila*, *X. bovienii*, *X. beddingii*, and *X. poinarii* contained megaplasmids (48 to >680 kb) and that there were no differences in megaplasmid profile between phases I and II.

The presence of lysogenic phage in and production of bacteriocins by *X. nematophila* was demonstrated by Boemare et al. (1992). Lysis of both phases of *X. nematophila*, *X. bovienii*, and *X. beddingii* in response to mitomycin C or heat shock released complete and partial phages. In addition to inhibiting nonhost *Xenorhabdus*, the bacteriocins inhibited *P. luminescens*, *Proteus vulgaris*, and *Morganella morganii* but no other Gram-negative or Gram-positive bacteria tested, indicating that the bacteriocins act against closely related genera in contrast to the antibiotics produced by phase I variants that have a wide spectrum of activity. The *X. nematophila* bacteriocin xenorhabdicin was shown to consist of two major protein bands, corresponding to the sheath and core, and five minor bands; the phage head capsid had one major and two minor subunits (Thaler et al., 1995).

Antibiograms scored after 3 d at 28°C show that *Xenorhabdus* spp. are inhibited by streptomycin, neomycin, gentamicin, tetracycline, kanamycin, and colistin, but not penicillin. Most strains are resistant to ampicillin and cephaloridine and, to a lesser extent, furazolidone, whereas resistance to chloramphenicol is limited. Resistance to streptomycin, tetracycline, and kanamycin after selection has been demonstrated for *X. nematophila*.

Xenorhabdus spp. are insect pathogens only when delivered into the insect hemocoel, either by their nematode symbiont or by injection; they are not pathogenic when applied topically. Most are highly toxic for larvae of the greater wax moth, *Galleria mellonella*, with an LD_{50} of <20 cells (Akhurst and Dunphy, 1993). *X. poinarii* has very little pathogenicity for *G. mellonella*, (LD_{50} = 5000 cells) when injected alone, although it is highly pathogenic when co-injected with axenic *Steinernema glaseri*, its natural host (Akhurst, 1986b). *X. nematophila* produces an orally active toxin complex of the same family as those produced by *Photorhabdus*, *Serratia entomophila*, and *Yersinia pestis* (Glare and Hurst, 2002) and a smaller single unit toxin that is toxic by injection (East et al., 1998). Their toxicity varies between insects, with *X. nematophila* having an LD_{50} of about 500 for *Hyalophora cecropia* caterpillars (Götz et al., 1981) and no toxicity to maggots of the genus *Chironomus* (Götz and Boman, 1985). *Xenorhabdus* spp. do not have any demonstrable effects on vertebrates (Poinar and Thomas, 1967; Poinar et al., 1982; Obendorf et al. 1983).

Xenorhabdus species have been found only in the intestinal tract of infective-stage nematodes of the genus *Steinernema* (syn. *Neoaplectana*) and in insects killed by these nematodes. There is a high degree of specificity in these associations, with each nematode species being naturally associated with only one *Xenorhabdus* species (Akhurst, 1983a; Table BXII.γ.278). This specificity is determined by the ability of the nematode to retain the bacterium in its intestinal vesicle (Bird and Akhurst, 1983; Akhurst and Boemare, 1990). Although there have been reports of other bacteria being associated with *Steinernema* (Lysenko and Weiser, 1974; Mracek, 1977; Aguillera et al., 1993), closer examinations have always demonstrated that only *Xenorhabdus* have a specific association (Akhurst, 1982b; Bonifassi et al., 1999).

The infective stage nematodes act as vectors, transporting the bacteria into the insect via natural orifices (mouth, anus, spiracles) and then into the hemocoel. The infective (dauer) nematode, a third-stage juvenile with closed mouth and anus, recom-

FIGURE BXII.γ.213. Phylogenetic tree showing the relationship of the genus *Xenorhabdus* to members of the *Gammaproteobacteria*. Note that since the publication of this figure, *Chromatium vinosum* has been transferred to the genus *Allochromatium* as *Allochromatium vinosum* (Imhoff et al., 1998b) and that *Vibrio marinus* has been transferred to the genus *Moritella* as *Moritella marina* (Urakawa et al., 1998, 1999b); in addition, the spelling of the species name *japonicus* has been corrected to *japonica* and that of *nematophilus* to *nematophila* (Euzéby and Boemare, 2000). (Reprinted with permission from T. Suzuki et al., Journal of Basic Microbiology, *5:* 351–354, 1996 ©Wiley-VCH Verlag, Berlin.)

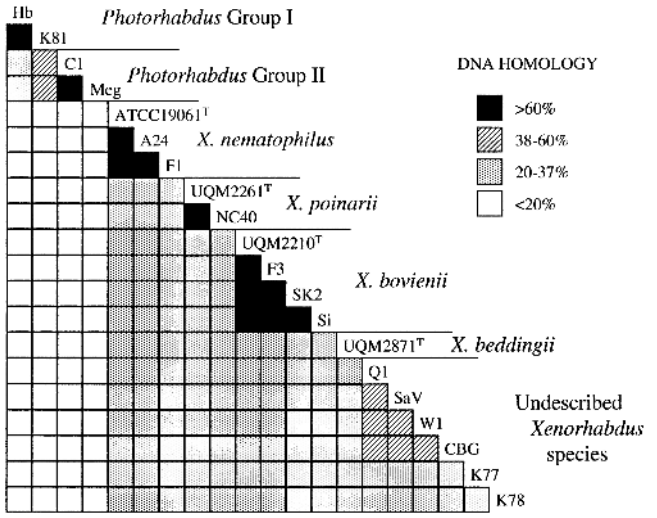


FIGURE BXII.γ.214. DNA relatedness of 20 strains of bacterial symbionts of entomopathogenic nematodes (modified from Boemare et al., 1993), indicating that strains representing a number of undescribed species have been isolated. In addition, the spelling of the species name *nematophilus* has since been corrected to *nematophila*.

mences development in the hemocoel, releasing its symbiotic bacterium and an inhibitor of the inducible antibacterial enzymes (Poinar and Himsworth, 1967; Götz et al., 1981). As *Xenorhabdus* multiplies it provides essential nutrients for nematode maturation and reproduction (Poinar and Thomas, 1966) and produces antibiotics (Paul et al., 1981; Akhurst, 1982a; McInerney et al. 1991a, b; Li et al., 1996).

Xenorhabdus alternates between a nutrient rich (insect) and nutrient poor (nematode) existence. Forst et al. (1997) hypothesized that phase II may be induced by the nematode gut conditions and better adapted to the nutrient poor conditions of the intestinal vesicle of the nonfeeding nematode. However, this hypothesis does not account for the fact that the bacteria isolated from field-collected infective stage juveniles are inevitably phase I. Smigielski et al. (1994) found differences in the activity of respiratory enzymes of phases I and II that indicate the greater potential of phase II to survive in soil environments than phase I. The lack of a record of isolation of *Xenorhabdus* directly from soil may be due to its slow growth, lack of a suitable selective medium, and/or the difficulty of identifying phase II, which has few positive characters in most standard tests for identifying bacteria. However, Morgan et al. (1997) found that *X. nematophila* declined very quickly in river water and soil, becoming undetectable after 2 d and 7 d, respectively. Although the phase status

TABLE BXII.γ.278. Biochemical characterization of the genus *Xenorhabdus*[a]

Characteristic[b]	1. *X. nematophila*	2. *X. beddingii*	3. *X. bovienii*	4. *X. japonica*	5. *X. poinarii*
Mol% G + C of DNA	43–48	45.5–50	44–47	45.9	42.6–49
Isolated from:					
S. carpocapsae	+	−	−	−	−
S. feltiae, S. intermedium, S. affine, S. kraussei	−	−	+	−	−
S. glaseri, S. cubanum	−	−	−	−	+
Unidentified *Steinernema* spp. (*Steinernema longicaudum*?)	−	+	−	−	−
S. kushidai	−	−	−	+	−
Pathogenicity for lepidopteran insects	+	+	+	−	−
Motility	d	d	d	d	d
Peritrichous flagella	+	+	+	+	+
Protoplasmic inclusions	d	d	d	nr	d
Bioluminescence	−	−	−	−	−
Pigmentation[c]	ow	lb	y	yb	br
Dyes adsorption	d	d	d	d	d
Antimicrobial production	d	d	d	d	d
Oxidation-fermentation	F	F	F	F	F
Catalase	−	−	−	−	−
Nitrate reduced to nitrite	−	−	[−]w	−	−
Oxidase, Kovac's	−	−	−	−	−
KCN, growth in	−	−	−	−	−
Indole production	−	−	−	−	−
Methyl red	−	−	−	−	−
Voges–Proskauer	−	−	−	−	−
Simmons citrate	+	+	+	−	+
Hydrogen sulfide production	−	−	−	−	−
ONPG (β-galactosidase)	−	−	−	−	−
Esculin hydrolysis	−	+	−	−	d
Urease, Christensen's	−	−	−	−	−
Phenylalanine deaminase	d	−	[−]	dw	[−]
Tryptophan deaminase	−	+w	+	−	−
Lysine decarboxylase	−	−	−	−	−
Ornithine decarboxylase	−	−	−	−	−
Arginine dihydrolase	−	−	−	−	−
D-Glucose, acid production	+	+	+	+	+
D-Glucose, gas production	−	−	−	−	−
Acid production from:					
D-Adonitol	−	−	−	−	−
L-Arabinose	−	−	−	−	−
Cellobiose	−	−	−	−	−
Dulcitol	−	−	−	−	−
Fructose	+	+	+w	+	+
Glycerol	+w	+	+	+	+
N-Acetylglucosamine	+	+	+	+	+
myo-Inositol	+w	−	dw	−	−
Lactose	−	−	−	−	−
Maltose	+	+	+	+	+
D-Mannitol	−	−	−	−	−
D-Mannose	+	+	+	+	+
Melibiose	−	−	−	−	−
α-Methyl-D-glucoside	−	−	−	−	−
Raffinose	−	−	−	−	−
L-Rhamnose	−	−	−	−	−
Ribose	−	+	+	−	−
Salicin	−	+	−	−	−
D-Sorbitol	−	−	−	−	−
Sucrose	−	−	−	−	−
Trehalose	+	+	+w	+	+
D-Xylose	−	−	−	−	−
Utilization of:					
Diaminobutane	−	−	[+]	−	−
L-Fucose	−	−	−	−	−
DL-Glycerate	+	+	+	−	[−]
L-Histidine	−	+	+	−	[+]
myo-Inositol	+w	−	dw	−	−
DL-Lactate	+	+	+	+	+
D-Mannitol	−	−	−	−	−
Ribose	−	+	[+]	−	−
L-Tyrosine	−	+	[+]	−	−
Gelatin hydrolysis, Kohn's	+	+	+	d	+
Lecithinase (egg yolk agar)	d	d	d	d	−
Lipase (Tween 80)	d	+w	+	−	+w
DNase	+	+	+	+	+w

[a]All tests were done at 28° ± 1°C for *Xenorhabdus*. Data from Akhurst (1986a,b); Boemare and Akhurst (1988); Yamanaka et al. (1992); Brehélin et al. (1993); Nishimura et al. (1994); Fischer-Le Saux et al. (1998).

[b]Symbols: +, 90–100% of strains are positive; [+], 76–89% are positive; d, 26–75% are positive; [−], 11–25% are positive; −, 0–10% are positive; w, weak reaction; nr, not reported; F, fermentative.

[c]ow, off-white; y, yellow; br, brown; lb, light brown; yb, yellowish brown.

of *X. nematophila* used in that study was not specified, it seems likely that they used phase I. At this time, there is no satisfactory explanation of the ecological role of phase II.

Enrichment and Isolation Procedures

Xenorhabdus spp. generally grow well at 25–28°C on nutrient or similar agar (e.g., Luria-Bertani, trypticase soy), but best growth has been achieved with yeast extract-salts (YS; Dye, 1968) and medium X (Götz et al., 1981) agars. *Xenorhabdus* can be isolated from the infective-stage nematodes by the hanging drop technique or by maceration (Poinar and Thomas, 1966; Akhurst, 1980). For both methods, the infective juveniles must first be surface sterilized; this is readily achieved by immersing a small number of live infective-stage nematodes (<100), free of debris, in 0.1% merthiolate for 1 h at room temperature and then rinsing thoroughly in several changes of sterile water. In the hanging-drop technique, individual surface-sterilized infective juveniles are transferred to a drop of aseptically collected insect hemolymph on a coverslip that is then inverted over a cavity to prevent desiccation and incubated at 25°C until the nematodes commence development (1–3 d). At this time, they void their symbiotic bacteria, which can be subcultured from the hemolymph onto an agar medium (e.g., nutrient agar) 1 d later. A more rapid method involves the maceration of 50–100 surface-sterilized infective juveniles in a nutrient broth by means of a tissue homogenizer. The macerate should be plated onto an agar medium immediately (10–100 µl aliquots) and incubated at 28°C for 3 d. The inclusion of suitable controls to confirm that the surface-sterilization procedure has been effective is essential for both methods. *Xenorhabdus* can also be isolated by the less labor-intensive method of collecting hemolymph from an insect (e.g., *G. mellonella*) larva within 24 h of its death after infection by *Steinernema*. With this last method, bacteria other than *Xenorhabdus* may also be isolated; these bacteria may be carried into the host on the exterior of the nematodes or may be picked up into the hemolymph from the insect cuticle. Contamination by other bacteria can be minimized by burying the insect in clean, damp sand, adding a small number of nematodes to the surface of the sand and incubating at 20–25°C until one to five nematodes infect the insect. This last method is better suited to reisolation of a *Xenorhabdus* strain rather than to determining the identity of the bacteria specifically associated with a nematode species.

Maintenance Procedures

Storage in 20% glycerol at −70°C or below is very useful for long-term maintenance of *Xenorhabdus* cultures; they do not store well at −20°C. Short-term storage (less than 1 month) is best conducted at 10–15°C because survival on agar or in broth at 4°C is very poor, and when cultures are maintained at temperatures in excess of 15°C there is a significant risk of a proportion of the culture undergoing phase change.

Procedures for Testing Special Characters

All tests for phenotypic characterization of *Xenorhabdus* spp. should be conducted at 28°C.

Dye adsorption in most *Xenorhabdus* species can be tested on nutrient agar containing 0.0025% (w/v) bromothymol blue and 0.004% (w/v) triphenyltetrazolium chloride (NBTA; Akhurst, 1980). Dye-adsorbing phase I colonies will appear dark blue, while nonadsorbent phase II colonies will be red. As *X. poinarii* does not adsorb bromothymol blue, dye adsorption in this species can be assessed on MacConkey agar; adsorbing colonies are dark red (Akhurst, 1986b). Congo red (Francis et al., 1993) and some other dyes can also be used for most *Xenorhabdus* spp.

Insect pathogenicity is tested by injection of 100, 1000, and 10,000 cells (total count) from a 24-h broth culture into final instar *G. mellonella* larvae. The injected larvae should be placed on dry filter paper in Petri dishes and incubated at 25°C for 3 d. Most *Xenorhabdus* spp. will kill >50% at a dosage of 100 cells; all will kill >50% at 10,000 cells. The use of a less susceptible host (e.g., *Manduca sexta*) has enabled detection of differences in pathogenicity between the two phases of a strain (Volgyi et al., 1998).

Antibiotic production is tested by spot inoculating *Xenorhabdus* spp. onto nutrient agar and incubating at 28°C for 3 d. At this time the bacteria are killed by exposure to chloroform vapor for ~1 h. After the chloroform has evaporated from the agar, semi-solid nutrient agar (0.5%) inoculated with a suitable indicator organism (*Micrococcus luteus* or another Gram-positive species) is poured to form a thin layer. After incubation at 28°C overnight, a halo of inhibition around antibiotic-producing colonies will be evident.

Differentiation of the genus *Xenorhabdus* from other genera

Xenorhabdus spp. are easily distinguished from other *Enterobacteriaceae* by the absence of catalase in the former and, with the exception of *Photorhabdus*, by their inability to reduce nitrate. The key characteristics for differentiating *Xenorhabdus* from its most closely related genera are shown in Table BXII.γ.279.

Taxonomic Comments

The first symbiotic bacterium isolated from entomopathogenic nematodes was described as a new species *Achromobacter nematophilus* (Poinar and Thomas, 1965). With the rejection of the genus *Achromobacter* (Hendrie et al., 1974), this new species could not be accommodated within any existing genus. Thomas and Poinar (1979) described a new genus, *Xenorhabdus*, to accommodate the bacterial symbionts of entomopathogenic nematodes as two species, *X. nematophila*, symbionts of the family *Steinernematidae*, and *"X. luminescens"*, associated with the *Heterorhabditidae*. These bacteria have low DNA–DNA relatedness (4%) to the type species of the type genus of the family *Enterobacteriaceae* (Farmer, 1984b) and lack nitrate reductase, which is positive for all other genera in the family. However, they do have the enterobacterial common antigen (Ramia et al., 1982), and phylogenetic analyses based on 16S rDNA (Suzuki et al., 1996; Brunel et al., 1997; Liu et al., 1997a; Szállás et al., 1997) confirm their relatedness to the *Enterobacteriaceae*.

From a phenotypic study of bacterial symbionts of the *Steinernematidae* and *Heterorhabditidae*, five groups were recognized

TABLE BXII.γ.279. Characteristics differentiating *Xenorhabdus*, *Photorhabdus*, and *Proteus*[a]

Characteristic	*Xenorhabdus*	*Photorhabdus*	*Proteus*
Bioluminescence	−	+	−
Catalase	−	+	+
Annular hemolysis	−	d	−
Urea hydrolysis	−	d	+
Indole	−	d	d
H_2S production	−	−	[+]
Nitrate reductase	−	−	+
Acid from mannose	+	+	−

[a]For symbols see standard definitions.

within the genus and the subdivision of *X. nematophila* into subspecies proposed (Akhurst, 1983b). A more comprehensive phenotypic study (Boemare and Akhurst, 1988) led to the elevation of the subspecies to species status, as *X. nematophila*, *X. bovienii*, *X. poinarii*, and *X. beddingii* (Akhurst and Boemare, 1988). *Xenorhabdus japonica*[1], symbiotically associated with *Steinernema kushidai*, was described later (Nishimura et al., 1994). DNA–DNA hybridization (Suzuki et al., 1990; Akhurst et al., 1996) and 16S rDNA (Suzuki et al., 1996; Brunel et al., 1997; Liu et al., 1997a; Szállás et al., 1997) analyses validated the inclusion of these five species in, and the exclusion of *P. luminescens* from, the genus.

DNA–DNA hybridization analysis indicates that there are more than the five *Xenorhabdus* species described to date (Fig. BXII.γ.214). Too few isolates of the other "species" have been characterized phenotypically to allow description of new species.

Further Reading

Bedding, R., R. Akhurst and H. Kaya. 1993. Nematodes and the Biological Control of Insect Pests, CSIRO, Melbourne.

Boemare, N.E., R.J. Akhurst and R.G. Mourant. 1993. DNA relatedness between *Xenorhabdus* spp. (*Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. Int. J. Syst. Bacteriol. *43*: 249–255.

Forst, S., B. Dowds, N.E. Boemare and E. Stackebrandt. 1997. *Xenorhabdus* and *Photorhabdus* spp.: bugs that kill bugs. Annu. Rev. Microbiol. *51*: 47–72.

Szallas, E., C. Koch, A. Fodor, J. Burghardt, O. Buss, A. Szentirmai, K.H. Nealson and E. Stackebrandt. 1997. Phylogenetic evidence for the taxonomic heterogeneity of *Photorhabdus luminescens*. Int. J. Syst. Bacteriol. *47*: 402–407.

List of species of the genus Xenorhabdus

1. **Xenorhabdus nematophila** (Poinar and Thomas 1965) Thomas and Poinar 1979, 355[AL] (*Achromobacter nematophilus* Poinar and Thomas 1965, 249.)
ne.ma.to' phi.la. Modern entomological term n. *nematode;* Gr. adj. *philus* loving or having affinity for; M.L. adj. *nematophila* nematode-loving.

   The species characteristics are listed in Tables BXII.γ.277 and BXII.γ.278. No isolates known to grow at temperatures in excess of 34°C. Neither phase is pigmented. Most isolates sensitive to furazolidone.

   Only found associated with one species of nematode, *Steinernema carpocapsae*, but distributed globally.

   The mol% G + C of the DNA is: 43–48 (Bd).

   Type strain: ATCC19061, DSM 3370.

   GenBank accession number (16S rRNA): D78009, X82251.

2. **Xenorhabdus beddingii** (Akhurst 1986a) Akhurst and Boemare 1993, 864[VP] (Effective publication: Akhurst and Boemare 1988, 1844) (*Xenorhabdus nematophilus* subsp. *beddingii* Akhurst 1986a, 456.)
bed.din' gi.i. M.L. gen. n. *beddingii* of Bedding, named for R.A. Bedding, who made significant contributions to the development of *Xenorhabdus/Steinernema* associations for insect pest control.

   The species characteristics are listed in Tables BXII.γ.277 and BXII.γ.278. All isolates grow at 34°C, some at 38°C. Hydrolyses esculin. Inhibited by cephaloridine and ampicillin. The brown pigmentation is not strong. Phase I species is highly unstable, producing the very stable phase II.

   Associated with two undescribed species of *Steinernema* from Australia, one of which may be *Steinernema longicaudum*, which was described from China.

   The mol% G + C of the DNA is: 45.5–50 (Bd, HPLC).

   Type strain: UQM 2871 (phase I of strain Q58), ATCC 49542, DSM 4764.

   GenBank accession number (16S rRNA): D78006, X82254.

3. **Xenorhabdus bovienii** (Akhurst 1983b) Akhurst and Boemare 1993, 864[VP] (Effective publication: Akhurst and Boemare 1988, 1843) (*Xenorhabdus nematophilus* subsp. *bovienii* Akhurst 1983b, 45.)
bo.vi.en' i.i. M.L. gen. n. *bovienii* of Bovien, named for P. Bovien who first reported the presence of bacteria in the intestinal vesicle of a *Steinernema* species.

   The species characteristics are listed in Tables BXII.γ.277 and BXII.γ.278. No growth at 34°C; some strains will grow at 5°C. Resistant to carbenicillin.

   Associated with several species of entomopathogenic nematode (*Steinernema feltiae*, *Steinernema intermedium*, *Steinernema kraussei*, *Steinernema affine*) in temperate regions.

   The mol% G + C of the DNA is: 44.3 (HPLC)–46.9 (Bd).

   Type strain: UQM2210 (phase I of strain T228), ATCC 35271, DSM 4766.

   GenBank accession number (16S rRNA): X82254, D78007.

4. **Xenorhabdus japonica** Nishimura, Hagiwara, Suzuki and Yamanaka 1995, 619[VP] (Effective publication: Nishimura, Hagiwara, Suzuki and Yamanaka 1994, 209.)
ja.po' ni.ca. M.L. adj. *japonica* of Japan.

   The species characteristics are listed in Tables BXII.γ.277 and BXII.γ.278. Does not grow at 37°C. Arginine dihydrolase activity detected in phase II. Pigmentation is yellowish brown.

   Only known to be associated with *Steinernema kushidai* in Japan.

   The mol% G + C of the DNA is: 45.9 (HPLC).

   Type strain: IAM 14265.

   GenBank accession number (16S rRNA): D78008, Z76739.

5. **Xenorhabdus poinarii** (Akhurst 1983b) Akhurst and Boemare 1993, 864[VP] (Effective publication: Akhurst and Boemare 1988, 1843 (*Xenorhabdus nematophilus* subsp. *poinarii* Akhurst 1983b, 45.)
poi.nar' i.i. M.L. gen. n. *poinarii* , of Poinar, named for G.O. Poinar, Jr., who made major contributions to the understanding of entomopathogenic nematode/bacterial interactions.

   The species characteristics are listed in Tables BXII.γ.277 and BXII.γ.278. This is the most heat tolerant *Xenorhabdus*, with all strains growing at 36°C and some at 40°C. The intensity of pigmentation in phase I varies from light to reddish brown. Antibiotic production by phase I and phase II is variable. Associated with *Steinernema glaseri* and *Steinernema cubanum* in the USA and Caribbean. The nematode/bacterium association does not appear to be as strong for

1. Euzéby and Boemare (2000) noted that *-rhabdus* has a feminine root and so species names for genera ending in *-rhabdus* should also be feminine.

this species as for other *Xenorhabdus*; its symbiosis may be more primitive. It is not pathogenic for greater wax moth (*Galleria mellonella*) larvae unless associated with its nematode partner.

The mol% G + C of the DNA is: 42.6 (HPLC)–49 (Bd).

Type strain: UQM 2216 (phase I of strain G), ATCC 35272, DSM 4768.

GenBank accession number (16S rRNA): D78010, X82253

Genus XLI. **Yersinia** *Van Loghem 1944, 15*[AL]

EDWARD J. BOTTONE, HERVÉ BERCOVIER AND HENRI H. MOLLARET

Yer.si'ni.a. M.L. fem. n. *Yersinia* named for the French bacteriologist A.J.E. Yersin, who first isolated the causal organism of plague in 1894.

Straight rods to coccobacilli, 0.5–0.8 × 1–3 μm. Endospores are not formed. Capsules are not present, but an envelope occurs in *Y. pestis* strains grown at 37°C and in cells from clinical sources. Gram negative, **nonmotile at 37°C, but motile with peritrichous flagella when grown below 30°C, except for *Y. pestis*, which is always nonmotile**. Growth occurs on ordinary bacteriological media. Colonies of yersiniae are translucent to opaque, 0.1–1.0 mm in diameter after 24 h. Optimum growth temperature, 28–29°C. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Oxidase negative. Catalase positive. Nitrate is reduced to nitrite with a few exceptions in specific biovars. D-glucose and other carbohydrates are fermented with **acid production but little or no gas**. **Phenotypic characteristics are often temperature dependent, and usually more characteristics are expressed by cultures incubated at 25–29°C than at 35–37°C.** The enterobacterial common antigen is present in all species investigated. Widely distributed in nature with some species adapted to specific animal hosts and humans. Several species are pathogenic for humans and animals including *Y. pestis*, the causative agent of plague. A significant cause of food-borne and waterborne disease. Belongs to the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 46–50.

Type species: **Yersinia pestis** (Lehmann and Neumann 1896) Van Loghem 1944, 15 (*Bacterium pestis* Lehmann and Neumann 1896, 194; *Yersinia pseudotuberculosis* subsp. *pestis* Bercovier, Mollaret, Alonso, Brault, Fanning, Steigerwalt and Brenner 1981a, 383.)

FURTHER DESCRIPTIVE INFORMATION

Cells of *Yersinia* species are small, coccoid-shaped Gram-negative bacilli that resemble cells of *Pasteurellaceae* rather than cells of *Enterobacteriaceae*. Pleomorphism occurs depending on the type of medium used and the temperature of incubation. Rods, coccobacilli, and small chains of four to five elements (especially in liquid media) can occur. Gram, Giemsa, or Wayson-stained smears reveal a more pronounced tendency to bipolar staining in *Y. pestis* than in the other species. Spores or specific inclusions are not formed. No definite capsule occurs, but *Y. pestis* displays a carbohydrate-protein envelope termed capsular antigen or fraction I (F-1) when cultured at 37°C (Burrows, 1963), or in direct smears taken from infected hosts (mice, guinea pigs, humans). L forms have been described for *Y. enterocolitica* (Pease, 1979).

Yersinia species are nonmotile after growth at 37°C but motile at 22–29°C, except *Y. pestis*, which is nonmotile irrespective of incubation temperatures. Fresh isolates of *Y. enterocolitica* and *Y. pseudotuberculosis* may require a few subcultures to express their motility. Motile cells have 2–15 peritrichous flagella characterized by a long wavelength (Nilehn, 1969).

Yersinias do not differ from other *Enterobacteriaceae* in their fine structure and overall cell wall composition. Lipopolysaccharides (O antigens) have been isolated and characterized (Davies, 1958; Rische et al., 1973). The whole-cell lipid composition of all *Yersinia* species investigated exhibits a pattern shared with other *Enterobacteriaceae* (Tornabene, 1973; Jantzen and Lassen, 1980). *Y. pestis* lipopolysaccharide has core components in common with other *Enterobacteriaceae* but lacks extended O-group side chains (Perry and Fetherston, 1997).

Placement of the genus *Yersinia* within the *Enterobacteriaceae* family is supported by both biochemical and DNA–DNA relatedness studies. Further studies (Ahmad et al., 1990) comparing rDNA sequence analysis to clustering based on aromatic amino acid synthesis placed the genus *Yersinia* in one of three "enteroclusters" along with *Cedecea*, *Edwardsiella*, *Hafnia*, *Kluyvera*, *Proteus*, *Providencia*, and *Morganella*. Sequence analysis of the 16S rRNA gene performed by Ibrahim and colleagues (1993) of representatives of 10 of the 11 *Yersinia* species reveal that yersiniae form a coherent cluster within the *Gammaproteobacteria* with sequence similarities ranging from 96.9–99.8%. Within the *Gammaproteobacteria* the closest relative is *Hafnia alvei* (96.5% sequence similarity).

Intragenerically, phylogenetic analysis disclosed five sublines, with *Y. enterocolitica*, *Y. rohdei*, and *Y. ruckeri* forming separate sublines. A separate subline was formed by *Y. pestis*, *Y. pseudotuberculosis*, and *Y. kristensenii*, while *Y. mollaretii*, *Y. intermedia*, *Y. bercovieri*, *Y. aldovae*, and *Y. kristensenii* formed a fifth subline.

Yersinia species grow on nutrient agar without enrichment. A small colony diameter differentiates yersiniae from all other *Enterobacteriaceae*. After incubation for 24–30 h at 30°C or 37°C, *Y. pestis* forms minute colonies (0.1 mm) that can be discerned only with difficulty by the naked eye. After 48 h their diameter increases to 1.0–1.5 mm. The colonies are slightly opaque, butyrous, smooth, round, and have somewhat irregular edges. The use of enriched media (serum, blood, yeast extract) does not dramatically improve growth, and after 48 h the colony sizes are similar to those found on nutrient agar. All other *Yersinia* species grown on nutrient agar at 25–37°C produce visible colonies in 24 h. The colonies reach a diameter of 1.0–1.5 mm after 24–30 h, and 2.0–3.0 mm after 48 h. After 18 h they are translucent, smooth, and round with irregular edges, but after 48 h the centers become elevated and the edges become more regular, producing a "Chinese hat" shape. *Y. pestis* colonies may appear slightly mucoid. When cultured for 48 h, all *Yersinia* species dissociate into small (0.5 mm) and large (2 mm) colonies. This phenomenon appears to depend on the medium used (Bercovier et al., 1979).

Growth is moderate in liquid media: incubation of *Yersinia* for 48 h will yield the same turbidity that occurs in 18 h with other *Enterobacteriaceae*. When grown in nutrient broth, clumps of *Y.*

pestis cells adhere to the side of the tube, which slowly forms a deposit at the bottom of the tube while the supernatant remains relatively clear; this is followed by the appearance of a pellicle, which in turn disintegrates to form flocculent masses and a larger deposit. This phenomenon is attenuated in peptone water. *Y. pseudotuberculosis* occasionally grows in a manner similar to that of *Y. pestis*. All other *Yersinia* species give uniform turbidity in nutrient broth and in peptone water.

Y. pestis and *Y. pseudotuberculosis* give variable growth responses on MacConkey agar. All other species grow well on this medium, with colonies reaching a size similar to that observed on nutrient agar. On Salmonella–Shigella agar incubated at 25°C, *Y. pestis* hardly grows at all, whereas all the other species produce pinpoint colonies in 24–30 h. When incubated on this medium at 37°C, *Y. enterocolitica* is only partially inhibited, whereas all other species are severely inhibited (Nilehn, 1969; Bottone, 1977; Bercovier et al., 1979).

All *Yersinia* species except *Y. pestis* can grow at 25°C on synthetic mineral-salt media with various carbohydrates as the energy source (Burrows and Gillett, 1966; Bercovier et al., 1979). *Y. pestis* requires L-methionine, L-isoleucine, L-valine, and L-phenylalanine, and or L-threonine for growth. When incubated at 37°C on synthetic mineral-salt media all *Yersinia* species become auxotrophic, and the addition of biotin and thiamine, and pantothenate and glutamic acid for *Y. pestis*, is necessary to promote growth (Burrows and Gillett, 1966; Brubaker, 1991). The growth of *Y. pestis* on such media is further enhanced by the addition of a reducing agent, and by incubation in a CO_2-enriched atmosphere (Brubaker, 1991). Virulent strains of *Y. pestis* require Ca^{2+} or ATP for growth at 37°C but not at 25°C (Zahorchak et al., 1979). This temperature-dependent requirement for Ca^{2+}, mediated by the Lcr plasmid, has also been described for virulent strains of *Y. pseudotuberculosis* and *Y. enterocolitica*.

The growth range is 4–42°C, with an optimum temperature of 28–29°C. *Y. pestis* and *Y. pseudotuberculosis* tolerate a pH range of 5.0–9.6; other *Yersinia* species can grow in a pH range of 4.0–10.0. The optimum pH for all species is 7.2–7.4. *Yersinia* species can grow in peptone water without the addition of NaCl. *Y. pestis* and *Y. pseudotuberculosis* tolerate up to 3.5% NaCl, and the other species can tolerate up to 5% NaCl. *Y. pseudotuberculosis*, but not *Y. enterocolitica*, which lacks tellurite reductase, is the only species that grows well on media containing 0.06% tellurite (Brzin, 1968).

Yersinia species do not differ significantly from other *Enterobacteriaceae* in their general metabolism (Brubaker, 1991) (Table BXII.γ.280). They produce acid during fermentation of D-glucose. *Y. enterocolitica*, *Y. frederiksenii*, and *Y. intermedia* produce acetoin (positive Voges–Proskauer test) when incubated at 28°C, whereas this characteristic is variable for *Y. ruckeri* and is absent in *Y. pestis* and *Y. pseudotuberculosis*. No *Yersinia* species produces acetoin at 37°C (Table BXII.γ.281).

The main physiological and biochemical characteristics of the various *Yersinia* species are given in Table BXII.γ.282. *Yersinia* ferment carbohydrates usually without gas production, a characteristic that is constant for *Y. pestis* and *Y. pseudotuberculosis*, but other species may produce a few bubbles after 2–3 days at 28°C. Because the optimum growth temperature of yersiniae is 28–29°C, some biochemical activities are often temperature-dependent (cellobiose and raffinose fermentation, ornithine decarboxylase, ONPG (*o*-nitrophenyl-β-D-galactopyranoside) hydrolysis, indole production, and the Voges–Proskauer reaction) and are more constantly expressed at 28°C rather than at 37°C. All species except *Y. intermedia* reduce nitrate to nitrite by a type B nitrate reductase; *Y. intermedia* strains have either a type A nitrate reductase, like most *Enterobacteriaceae*, or a type B reductase. ONPG activity of yersiniae does not correspond to a true β-galactosidase, but only to an ONPG-ase (Le Minor and Coynault, 1976). In addition to the characteristics given in Tables BXII.γ.281 and BXII.γ.282, *Yersinia* species are able to attack polypectate in 5–7 days and starch in 3–7 days. Yersiniae are neither hemolytic nor proteolytic, except *Y. ruckeri*, which liquefies gelatin, and some strains of *Y. pestis*, which have fibrinolytic and coagulase activity linked to the production of Pesticin 1. Plasminogen activator coagulase activity and pesticinogeny are plasmid (pPCD1) encoded. Lecithinase activity in *Y. enterocolitica* is strain-dependent. *Y. pseudotuberculosis*, *Y. enterocolitica*, and *Y. ruckeri* strains have a lipase that is active on corn oil, but only *Y. intermedia*, *Y. frederiksenii*, and *Y. enterocolitica* biovar I express a lipase-esterase that is active on Tween 80.

Transformation of auxotrophic strains of *Y. enterocolitica* by prototrophic strains using the Juni-Janik technique has been reported (Callahan and Koroma, 1979). F lac+ episomes from *E. coli* have been transferred to *Y. pestis* (Martin and Jacob, 1962), to *Y. pseudotuberculosis* (Lawton et al., 1968b), and to *Y. enterocolitica* (Cornelis and Colson, 1975), but usually with a low frequency (10^{-4}–10^{-6}) . This has allowed chromosomal mapping of *Y. pseudotuberculosis* (Lawton and Stull, 1971; McMahon, 1973). Gene transfer by conjugation between *Y. pseudotuberculosis* and *Y. pestis* has also been demonstrated (Lawton et al., 1968a).

R factors have been transferred to *Y. pestis* and *Y. pseudotuberculosis* (Ginoza and Matney, 1963) and to *Y. enterocolitica* (Knapp and Lebek, 1967). Wild strains of *Yersinia* carrying R plasmids (Cornelis et al., 1973; Kanazawa and Ikemura, 1979) appear to be rare. This could be explained, at least for *Y. enterocolitica*, by the presence of a restriction-modification system (Cornelis and Colson, 1975). A self-transmissible plasmid coding for lactose fermentation has been described in *Y. enterocolitica* (Cornelis et al., 1976). This plasmid is freely transmissible between strains of *Y. enterocolitica* and *E. coli*.

Other plasmids related to various virulence tests (Ca^{2+} dependency, autoagglutination, lethality for mice and gerbils, Sereny test) have been demonstrated in *Y. pestis* (Ferber and Brubaker, 1981; Brubaker, 1991), *Y. pseudotuberculosis* (Gemski et al., 1980b), and *Y. enterocolitica* (Gemski et al., 1980a; Zink et al., 1980). These plasmids of 40–48 MDa molecular weight constitute a family of related plasmids (Ben-Gurion and Shafferman, 1981; Portnoy et al., 1981). *Y. pestis* and *Y. pseudotuberculosis* have never been found to be lysogenic, whereas of 1252 strains of *Y. enterocolitica* studied, 86.4% were lysogenic when grown at 25°C but not at 37°C (Nicolle et al., 1973). Phages active on *Y. pestis* and *Y. pseudotuberculosis* have been described (Gunnison et al., 1951; Girard, 1953), but they are not host-specific and are used only for presumptive bacteriological diagnosis. Coliphages T2, T3, and T7 are also active on *Y. pseudotuberculosis* and *Y. pestis* (Hertman, 1964). A phage typing system, useful in epidemiological investigations has been developed for *Y. enterocolitica* (Nicolle et al., 1973): strains of *Y. enterocolitica* serogroup 0:3 are associated with phagovar VIII in Europe, IXa in the Republic of South Africa, and lXb in Canada and the United States (Bottone, 1997).

Strains of *Y. pestis* produce a bacteriocin active on *Y. pseudotuberculosis* (Ben-Gurion and Hertman, 1958). This was named Pesticin I by Brubaker and Surgalla (1962) after they detected a second bacteriocin (Pesticin 11) that was produced by *Y. pestis* and *Y. pseudotuberculosis*. Pesticin I has a narrow host range, being

TABLE BXII.γ.280. Differentiation of *Yersinia* from other genera[a]

Test	*Yersinia*	*Citrobacter*	*Enterobacter*	*Escherichia*	*Hafnia*	*Klebsiella*	*Proteus*	*Salmonella*
Voges–Proskauer	−	−	+[b]	−	[+]	d	d	−
Citrate (Simmons)	−	+	+	[−]	−	d	d	+
H_2S production	−	d	−	−	−	−	d	+
Phenylalanine deaminase	−	−	−	−	−	−	+	−
Lysine decarboxylase	−[c]	−	[−][d]	[+]	+	[+]	−	+
Motility, 37°C	−	+	+[b]	[+]	[+]	−	+	+
Motility, 25°C	+[e]	+	+[b]	[+]	[+]	−	+	+
KCN, growth	−[c]	d	+[f]	−[g]	+	+	+	-[h]
Malonate utilization	−	d	+[i]	d	d	+[j]	−	d
D-Glucose, gas	− or W	+	+[k]	+	+	[+]	[+]	+
L-Arabinose, acid	+[l]	+	+	+	+	+	−	+
D-Mannitol, acid	+[m]	+	+	+[n]	+	+	−	+
Mucate, acid	−	+	d	d	−	+[o]	−	+[p]

[a]For symbols see standard definitions; W, weak.

[b]Except *E. asburiae*.

[c]Except some strains of *Y. ruckeri*.

[d]*E. aerogenes* and *E. gergoviae* are positive.

[e]Except *Y. pestis* and some strains of *Y. ruckeri*.

[f]Except *E. gergoviae* and some strains of *E. agglomerans*.

[g]Except *E. hermannii* and a few strains of *E. vulneris*.

[h]Except *S. bongori* and some strains of *S. choleraesuis* subsp. *houtenae*.

[i]Except *E. asburiae* and *E. sakazakii*.

[j]Except *K. ozaenae*.

[k]Except *E. agglomerans*.

[l]Except *Y. ruckeri* and some strains of *Y. aldovae*, *Y. kristensenii*, and *Y. pseudotuberculosis*.

[m]Except some strains of *Y. aldovae*.

[n]Except *E. blattae*.

[o]Except *K. ozaenae* and *K. rhinoscleromatis*.

[p]Except *S. choleraesuis* subsp. *diarizonae* and *S. choleraesuis* subsp. *houtenae*.

active against a few strains of *E. coli*, serogroup IA and IB *Y. pseudotuberculosis* (Burrows, 1963), and serogroup 0:8 *Y. enterocolitica* (Brubaker, 1991). *Y. pestis* strains that produce Pesticin I also elaborate a fibrinolytic factor and a coagulase (Brubaker, 1972). A bacteriocin-like activity associated with the presence of phage tails has been described in *Y. enterocolitica* (Nicolle et al., 1973). *Y. intermedia* produces a bacteriocin-like substance at 25°C but not at 37°C that is active on certain strains of *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. kristensenii* (Bottone et al., 1979). Cafferkey et al. (1989) have shown that bacteriocin production by "avirulent" strains of serogroup 0:3 occurs at both 25°C and at 37°C and is active against isolates of serogroups 0:3, and single strains tested of 0:8 and 0:9 irrespective of the presence of the virulence plasmid.

The antigenic structure of *Yersinia* species is complex, but antigens are shared by *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. The common enterobacterial antigen has been found in these species (Le Minor et al., 1972; Maeland and Digranes, 1975). The fraction I envelope antigen (FI) of *Y. pestis* is best produced when cultures are incubated at 37°C on protein-rich media (Fox and Higuchi, 1958). This antigen, a large gel-like capsule or envelope, is heat labile (10 min at 100°C), water soluble, and dissociates during growth into a glycoprotein (FIA) and a carbohydrate-free protein (FIB). Passive hemagglutination with FI antigen is used for serologic surveys in plague foci. The presence of this antigen has also been demonstrated in *Y. pseudotuberculosis* (Quan et al., 1965). V and W antigens expressed by virulent strains of *Y. pestis* cultivated at 37°C appear to be related to the presence of a 45 MDa plasmid (Ben-Gurion and Shafferman, 1981; Ferber and Brubaker, 1981). Production of plasmid-mediated V and W antigens has also been described in *Y. pseudotuberculosis* (Gemski et al., 1980b) and in *Y. enterocolitica* (Gemski et al., 1980a). The somatic antigen of *Y. pestis* is rough (R antigen) and therefore no serogroups have been described in this species. This R antigen is also present in *Y. pseudotuberculosis* (Thal and Knapp, 1971). In addition, *Y. pestis* and *Y. pseudotuberculosis* share at least 11 of 18 antigens (Lawton et al., 1960). *Y. pestis* and *Y. enterocolitica* express common protein antigens (Barber and Eylan, 1976). The antigenic scheme for *Y. pseudotuberculosis* (Thal and Knapp, 1971) comprises eight main thermostable serogroups (I to VIII) with nine subtypes (IA, IB; IIA, IIB, IIC; III; IVA, IVB; VA, VB; VI; VII; VIII), and five thermolabile flagellar H antigens (a–e). Antigenic relationships have been demonstrated between *Y. pseudotuberculosis* (serogroups 11, IV, IVA, and VI) and *Salmonella* serogroups B and D, *E. coli* serogroups 017, 055, and 077, and *Enterobacter cloacae* (Knapp, 1968; Mair and Fox, 1973).

Wauters et al. (1972) initially described 34 different O antigen and 20 H antigen serogroups in *Y. enterocolitica*. This classification included some serogroups defined by strains belonging to *Y. intermedia* (O:17) and *Y. kristensenii* (O:11, 0:12, 0:28). Wauters et al. (1991) subsequently expanded the O and H antigenic schema to include approximately 60 serogroups. Serogroup expansion has also shown sharing of antigens between pathogenic and nonpathogenic *Y. enterocolitica* and *Y. bercovieri* (O:8), *Y. frederiksenii* (O:3), and *Y. mollaretii* (O:3). Nevertheless, serogrouping in conjunction with biogrouping (Table BXII.γ.283), is a useful epidemiological marker. Cross-reactions occur between *Y. enterocolitica* serogroup O:9 and *Brucella* species (Hurvell and Lindberg, 1973; Corbel, 1975).

Yersinia species are susceptible *in vitro* to the following anti-

TABLE BXII.γ.281. Reactions of *Yersinia* spp. at 25–28°C and 37°C[a]

Test	1. *Y. pestis*	2. *Y. aldovae*	3. *Y. bercovieri*	4. *Y. enterocolitica*	5. *Y. frederiksenii*	6. *Y. intermedia*	7. *Y. kristensenii*	8. *Y. mollaretii*	9. *Y. pseudotuberculosis*	10. *Y. rohdei*	11. *Y. ruckeri*
Voges–Proskauer:											
37°C	−	−	−	−	−	−	−	−	−	−	−
25-28°C	−	+	−	+	+	+	−	−	−	−	−
Citrate, Simmons:											
37°C	−	−	−	−	[−][b]	−	−	−	−	−	−
25-28°C	−	d	−	−	d	−	−	−	−	[+][c]	−
Urease:											
37°C	−	[+]	d	[+]	[+]	[+]	[+]	[−]	+	d	−
25-28°C	−	+	+	+	+	+	+	+	+	d	−
Ornithine decarboxylase:											
37°C	−	d	[+]	+	+	+	+	[+]	−	[−]	+
25-28°C	−	+	+	+	+	+	+	+	−	[+]	+
Motility:											
37°C	−	−	−	−	−	−	−	−	−	−	−
25-28°C	−	+	+	+	+	+	+	+	+	+	[+]
Glycerol, acid:											
37°C	d	−	−	+	[+]	d	d	[−]	d	d	d
25-28°C	d	+	[+]	+	+	+	+	+	+	[+]	d
myo-Inositol, acid:											
37°C	−	−	−	d	[−]	[−]	[−]	−	−	−	−
25-28°C	−	+	d	d	−	[+]	d	d	−	−	−
Melibiose, acid:											
37°C	[−]	−	−	−	−	[+]	−	−	d	d	−
25-28°C	d	−	−	−	−	+	−	−	+	d	−
Raffinose, acid:											
37°C	−	−	−	−	d	d	−	−	[−]	d	−
25-28°C	−	−	−	−	−	+	−	−	[−]	d	−
L-Rhamnose, acid:											
37°C	−	−	−	−	+	+	−	−	d	−	−
25-28°C	−	+	−	−	+	+	−	−	+	−	−
Salicin, acid:											
37°C	d	−	[−]	[−]	+	+	[−]	[−]	[−]	−	−
25-28°C	[+]	+	−	[−]	+	+	−	[−]	d	−	−
D-Xylose, acid:											
37°C	+	d	+	d	+	+	[+]	d	+	d	−
25-28°C	+	+	+	d	+	+	+	+	+	+	−
Mucate, acid:											
37°C	−	−	−	−	−	−	−	−	−	−	−
25-28°C	−	d	+	−	[−]	d	−	+	−	−	−
Esculin hydrolysis:											
37°C	d	−	[-]	[-]	[+]	[−]	−	−	+	−	−
25-28°C	+	+	[+]	d	+	+	−	[−]	+	−	−

[a]For symbols see standard definitions.

[b][-], 11–25% positive.

[c][+], 26–75% positive.

microbial agents: tetracycline, chloramphenicol, aminoglycosides (amikacin, streptomycin, gentamicin, kanamycin, and neomycin), sulfonamides (alone or in combination with trimethoprim), imipenem, aztreonam, and fluoroquinilones (Hoogkamp-Korstanje, 1987; Bonacorsi et al., 1994). They are variably susceptible to colistin and are resistant to erythromycin and novobiocin. *Y. pestis* and *Y. pseudotuberculosis* are usually susceptible to β-lactam antibiotics, but their susceptibility to penicillin is in the range of susceptible to intermediate. Resistance to ampicillin (Borowski and Zaremba, 1973) and to streptomycin (Kanazawa and Ikemura, 1979) has been described for *Y. pseudotuberculosis*, *Y. enterocolitica*, and *Y. intermedia* (Bottone, 1977). *Y. frederiksenii* and *Y. kristensenii* are resistant to penicillin and slightly susceptible or resistant to other β-lactam antibiotics (ampicillin, carbenicillin, cephalothin) (Bercovier et al., 1979). The level of resistance is strain dependent (Zaremba and Aldová, 1979) and temperature dependent (Chester and Stotzky, 1976). Antibiotic susceptibility patterns of *Y. enterocolitica* are serogroup specific. *Y. enterocolitica* strains produce both a constitutive β-lactamase (active on ampicillin, carbenicillin, penicillin, and cephalosporins) and an inducible β-lactamase active only on cephalosporins and penicillin (Cornelis and Abraham, 1975). *Y. enterocolitica* strains that are resistant to tetracycline, chloramphenicol, streptomycin, and kanamycin have been reported (Zaremba and Aldová, 1979). Newer β-lactam antibiotics such as ceftriaxone, ceftazidime, cefotaxime, and moxalactam have excellent activity against *Y. enterocolitica*, as do imipenem and aztreonam (Hornstein et al., 1985).

Y. pestis is the causative agent of plague. Plague is primarily a disease of wild rodents. *Y. pestis* is transmitted among wild rodents by flea bites or ingestion of contaminated animal tissues (Butler, 1983). In fleas, the bacterium multiplies and blocks the esophagus and the pharynx. The fleas regurgitate the bacteria when they take their next blood meal and transmit *Y. pestis* to humans if no other hosts are available. Infective flea bites produce the typical bubonic form of plague within 2–8 d. *Y. pestis* multiplies intracellularly in macrophages and extracellularly and proceeds through the lymphatic system. The lymph nodes near the flea

TABLE BXII.γ.282. Differential characteristics of the species of the genus *Yersinia*

Test[a]	1. *Y. pestis*	2. *Y. aldovae*	3. *Y. bercovieri*	4. *Y. enterocolitica*	5. *Y. frederiksenii*	6. *Y. intermedia*	7. *Y. kristensenii*	8. *Y. mollaretii*	9. *Y. pseudotuberculosis*	10. *Y. rohdei*	11. *Y. ruckeri*
Pathogenic	+	(+)[b]	(+)	+	(+)	(+)	(+)	(+)	+	(+)	(+)
Mol% G + C content	46	46	50	48.5 ± 0.5	48	48.5 ± 0.5	48.5 ± 0.5	50–51	46.5	48.7–49.4	48.0 ± 0.5
Motility (22°C)	−[c]	−	−	+	−	+	−	−	+	−	−
Nitrate reductase	V[d,e]	+	+	+	+	+	+	+	+	+	+
Urease	−[c]	−	d	+	+	+	+	−	+	d	−
Simmons citrate	−	d	−	−	V	+22°C or −	−	−	−	+	−
Ornithine decarboxylase	−	d	+	+	+	+	+	+	−	+	+
Acetylmethyl-carbinol (22°C)	−	+	+	+	+	+	−	+	−	+	+
B-Galactosidase	d	−	−	+	+	+	d	−	d	d	d
Indole	−	−	−	V	+	+	V	−	−	−	−
H_2S production	−	−	−	−	−	−	−	−	−	−	−
Aesculin hydrolysis	+	−	−	V	+	+	−	−	+	−	−
Gelatin	−	−	−	−	−	−	−	−	−	−	−
Methyl red	+[e]	+[d]	V	V	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	−	−	−	−	d	d	−	d	−	−	−
Sucrose	−	−	+	+	+	+	−	+	−	+	−
Glycerol	V[d]	−	−	+	+	d	d	−	V	d	d
α-Methylglucoside	−	−	−	−	−	+ (22°C)	−	−	−	−	−
Cellobiose	−	d	+	+	+	+	+	+	−[c]	−	−
Melibiose	V[d]	−	−	−	−	22°C[c]	−[c]	−	+	d	−
Mucate	−	d	+	−	V	V	−	+	−	−	−
Raffinose	−	−	−	−	d	d	−	−	−	d	−
L-Rhamnose	−	−	−	−	d	d	−	−	+[c]	−	−
Trehalose	+	+	+	V	V	+	−	+	+	+	+
Sorbose	−	NR[f]	NR	V	+	+	+	NR	−	NR	NR
Sorbitol	d	d	+	+	+	+	+	+	−[c]	+	d
Arabinose	+	+	+	+	+	+	+	+	+	+	−
Maltose	V[d]	−	+	+	+	+	+	d	+	−	+
D-Xylose	+	d	+	+	+	+	+	d	+	d	−
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Pyrazinamidase	−	−	V	V	+	+	+	−	−	+	+
Usual habitat:											
Animals				+	+		+		+		+
Birds									+		
Dogs										+	
Fish		+			+	+					+
Fleas	+										
Mammals	+										
Rodents	+					+				+	
Humans	+	+	+	+		+		+	+	+	+
Environment				+			+				
Soil			+				+				
Sewage						+					
Water		+			+	+				+	+
Food					+			+			+
Vegetables			+					+			

[a]All *Yersinia* spp. are fermentative; negative for H_2S, oxidase, arginine dihydrolase, phenylalanine deaminase, malonate, dulcitol, DNase (25°C), and pigmentation; all are positive for mannitol and mannose. Motile species have peritrichous flagella, and motility is more pronounced at temperatures less than or equal to 30°C. Except for delayed reactions in some *Y. ruckeri*, all species are negative for gelatin hydrolase and lysine decarboxylase.

[b](+), Opportunistic pathogens.

[c]Key test to differentiate species.

[d]Key test to differentiate biovars.

[e]V, Strain instability or variable.

[f]NR, Not reported.

bite are the first to become inflamed, enlarged, and painful, and constitute the bubo. As the evolution of the infection is usually rapid with massive growth of *Y. pestis* in the blood, characteristic lesions are not found in the spleen or liver at autopsy. Untreated, the disease evolves in 5–10 d to profound septicemia in which *Y. pestis* may be seen in peripheral blood smears (Mann et al., 1984). Secondary pneumonia may result from hematogenous spread. From the latter, primary pneumonic plague can spread

TABLE BXII.γ.283. Differentiation of biogroups of *Yersinia enterocolitica*[a]

Test	Biogroup Reaction					
	1A	1B[b]	2	3	4	5
Lipase activity	+	+	−	−	−	−
Salicin (acid production in 24 h)	+	−	−	−	−	−
Esculin hydrolysis (24 h)	±	−	−	−	−	−
Xylose (acid production)	+	+	+	+	−	V
Trehalose (acid production)	+	+	+	+	+	−
Indole production	+	+	V	−	−	−
Ornithine decarboxylase	+	+	+	+	+	+(+)
Voges–Proskauer test	+	+	+	+	+	+(+)
Pyrazinamidase activity	+	−	−	−	−	−
Sorbose (acid production)	+	+	+	+	+	−
Inositol (acid production)	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	−

[a]Symbols: +, positive; −, negative, (+), delayed positive; V, variable.

[b]Biogroup 1B is comprised mainly of strains isolated in the United States.

from person to person or from animals to humans by means of droplets. Both bubonic and pneumonic plague have been transmitted to humans by the domestic cat—bubonic through a scratch and bite from an infected cat (Thornton et al., 1975; Weniger et al., 1984), and pneumonic through face-to-face exposure to a cat with pneumonic plague (Werner et al., 1984; Doll et al., 1994). In this clinical form, death generally occurs in less than 4 d. Pestis minor cases, in which the bacteria remain self-limited in buboes followed by self-cures have been described in endemic plague areas (Pollitzer, 1954).

Virulence of *Y. pestis* is associated with several factors (Surgalla et al., 1968; Perry and Fetherston, 1997). Among these are synthesis of the fraction I (FI) gel-like antiphagocytic capsule or surface antigen, VW antigens (associated with Ca^{2+} dependency for growth on magnesium oxalate medium, autoagglutination in broth cultures at 37°C, pigment production (incorporation of Congo red dye or hemin into cell surfaces, which results in dark greenish-brown or red colonies at 26°C but not at 37°C), presence of siderophore (yersiniabactin) iron-acquisition system, pH6 antigen, a fibrillar adhesin surface structure that may facilitate entry into macrophages (Straley, 1993) and is synthesized in host macrophage lysosomes (Lindler and Tall, 1993), and serum resistance necessary for growth in blood and transmission between insect vector and mammalian hosts. Pesticin production (Pst plasmid) is necessary for dissemination of *Y. pestis* from peripheral sites of inoculation; Pst is a 9.5-kb plasmid that also encodes plasminogen activator, a fibrinolysin, coagulase activity, which may be limited to rabbit plasma (Brubaker, 1991), and murine toxin, a 6-adrenergic antagonist that is highly lethal for mice and rats, causing circulatory collapse. LD_{50} dose for mice inoculated with strains expressing the aforementioned virulence factors is 1–10 organisms. Avirulent strains of *Y. pestis* never produce VW antigens except in the case of the vaccine strain EV76, whose attenuated virulence has resulted from a mutation in its iron metabolism. Virulent strains and the EV76 strain harbor a 45-MDa plasmid. In contrast to the VW antigens, the lack of any of the other virulence factors does not completely abolish the virulence of *Y. pestis* strains.

Y. pseudotuberculosis is responsible for epizootics in nearly all animal species, especially in rodents and birds. Animals are usually contaminated by the oral route and, after 1–2 weeks of incubation, the bacteria are found in mesenteric lymph nodes. The main symptoms are mesenteric adenitis and chronic diarrhea. Infection evolves either in self-cure, or in fatal septicemia. *Y. pseudotuberculosis* is an intracellular parasite and, like *Y. pestis*, reaches the lymphatic system. At autopsy, caseous lesions are found in Peyer's patches, mesenteric lymph nodes, the spleen, and the liver. Humans orally contaminated by *Y. pseudotuberculosis* develop either a mesenteric adenitis, which simulates acute appendicitis, or, in the compromised host, a severe septicemia. *Y. pestis* and *Y. pseudotuberculosis* share a common 70-kb plasmid low calcium response (LCR) that encodes for calcium dependency and four outer membrane proteins, and the V and W antigens. *Y. pseudotuberculosis* chromosomally controlled virulence factors include the outer membrane protein invasin, which promotes host cell penetration (Isberg and Falkow, 1985).

Y. enterocolitica has been recognized as pathogenic for chinchillas, hares, monkeys, and humans. The pathogenicity for animals is similar to that of *Y. pseudotuberculosis*. In humans the most common presentation is acute enteritis in children. Concurrent mesenteric lymphadenitis and terminal ileitis simulating appendicitis may also be present. In young adults, acute terminal ileitis and mesenteric lymphadenitis appear to be more common. The extent of gastrointestinal tract pathology depends on the serogroup of the invading strain and age and underlying status of the host. In adults secondary clinical forms of infection include arthritis and erythema nodosum. Septicemia with metastatic abscesses is a rarer complication and is usually associated with immunosuppression or occurs in the setting of iron overload in individuals receiving the iron chelator desferrioxamine (Robins-Browne and Pipic, 1985). Septicemia and profound shock has been associated with transfusion of *Y. enterocolitica* contaminated blood (reviewed by Bottone, 1999). Secondary immunologically mediated sequelae include arthritis and erythema nodosum. Arthritis is closely associated with the presence of the histocompatibility antigen HLA-27 (Dequeker et al., 1980), especially among Scandinavians, and *Y. enterocolitica* serogroup 0:3 biotype 4, phage type 8, and serogroup 0:9 infections. Production of a heat-stable enterotoxin (ST) resembling *E. coli* ST (Okamoto et al., 1981) has been demonstrated *in vitro* (Pai and Mors, 1978), but its role in pathogenicity is not clear: *Y. enterocolitica* strains do not produce ST when incubated *in vitro* at temperature above 30°C, and direct proof of ST production *in vivo* has not been reported.

Virulent plasmid-containing strains of *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* rapidly become avirulent when grown at 37°C, which results in the loss of the virulence plasmid. Cross-immunity among these three species has been demonstrated

(Thal, 1973; Alonso et al., 1978). Human chemoprophylaxis with streptomycin (drug of choice), tetracycline, and chloramphenicol; vaccination; and the spreading of insecticides and rodenticides are the suggested measures for controlling plague. For persons exposed to *Y. pestis*, tetracycline, doxycyline, and trimethoprim-sulfamethoxazole are recommended (Poland, 1989).

The pathogenicity of *Y. intermedia*, *Y. kristensenii*, *Y. frederiksenii*, *Y. bercovieri*, and *Y. mollaretii* in humans and animals is not clearly established. They appear more like opportunistic pathogens than true pathogens (Bercovier et al., 1978; Wauters et al., 1988; Bottone, 1997). ST-producing strains of these three species have been described (Kapperud, 1980), but their clinical significance is still unknown. *Y. ruckeri* is a fish pathogen responsible for red mouth disease, especially in rainbow trout. An inflammation of the mouth and the throat is the main characteristic of the disease, which is enzootic (Rucker, 1966). The bacterium is usually isolated from the kidneys of fish undergoing a systemic infection.

Epidemiology The geographical distribution of *Y. pestis* is widespread, and the organism has been isolated from all continents except Australia and Antarctica. Plague is enzootic in Africa (Central, East, and South Africa), in North and South America, certain regions of Asia (Southeast Asia, Mongolia), and the former USSR, India, and southwestern United States and the Pacific coastal area (Perry and Fetherston, 1997). Between epidemics, *Y. pestis* remains localized in enzootic or maintenance hosts (Butler, 1983) and has been isolated from more than a hundred different naturally infected species of rodents, but rarely from predatory animals (carnivores and birds, the latter being resistant to the infection). The spread of plague is usually accomplished by the epizootic cycle of rodents to fleas and fleas to rodents. The reservoir for *Y. pestis* is soil contaminated by infected dead fleas and rodents in which the microorganism survives for months in deep rodent burrows.

Rodents coming from noninfected areas become infected when they dig burrows in previously contaminated areas (Mollaret et al., 1963). This cycle constitutes "sylvatic plague". When urban rodents come in contact with rural rodents, *Y. pestis* can spread between rodents and to humans through flea bites. The epidemiology of plague is thus linked to the ecology of both fleas and rodents.

Y. pseudotuberculosis is distributed worldwide. It has been found in numerous animal species, especially rodents and birds, in soil, and in humans (Wetzler, 1970). In Japan, cats and dogs have been associated with human cases (Fukushima et al., 1989). Wild animals, which are often asymptomatic carriers, are considered the reservoir of the bacteria. Humans and animals are contaminated orally either by direct contact with sick or asymptomatic animals or through food contaminated by the excretions of these animals. The incidence of *Y. pseudotuberculosis* infection varies with the season and is highest during colder months. *Yersinia* species multiply at 4°C and therefore have a selective advantage over other bacteria at low temperatures; this explains why *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. frederiksenii*, and *Y. kristensenii* are more frequently isolated from the environment during cold rather than hot seasons. Human and animal infections follow this seasonal distribution as well.

Y. enterocolitica has been isolated from a wide variety of sources (live and inanimate) in every country in which it has been sought and probably has a worldwide distribution (Mollaret et al., 1979). As shown in Table BXII.γ.284, biovar IA strains are ubiquitous, having been found in a wide range of animal and environmental sources (including foods), whereas other biovars or serogroups are frequently associated with a specific host (Bercovier et al., 1980a): biovar 5 strains have been isolated mainly from hares in Europe; biovar 4, serogroup 0:3 strains are responsible for most human gastrointestinal infections in Europe, Canada, and the Republic of South Africa, and recently in the United States as well (Bottone et al., 1987; Ostroff, 1995). Serogroup 0:8, heretofore the most frequently isolated *Y. enterocolitica* strain in the United States, has decreased in frequency in the United States, and is only sporadically reported in other parts of the world (Ostroff, 1995). Serogroup 0:9 strains are the second most common *Y. enterocolitica* isolates in Europe and Japan.

Y. intermedia and *Y. frederiksenii* have been identified in Europe, the United States, Australia and New Zealand, Israel, and Japan. These two species have been isolated mainly from fresh water and foods and only rarely from nonirrigated soil, humans or animals other than fish (Kapperud, 1977; Bercovier et al., 1978; Brenner et al., 1980a; Ursing et al., 1980a). *Y. kristensenii* has been found in Europe, the United States, Japan, and Australia. Strains of this species have been isolated mainly from soil, foods, and asymptomatic animals; isolates from other environmental sources and from human infection are rare (Bottone and Robin, 1977; Bercovier et al., 1980b). *Y. bercovieri* has been recovered from terrestrial ecosystems (Bercovier et al., 1978), while *Y. mollaretii* has been isolated from human stool specimens, most raw vegetables, soil, and drinking water (Fukushima, 1985; Kaneko and Maruyama, 1987). Evidence of human disease potential for either *Y. bercovieri* or *Y. mollaretii* are lacking.

Y. ruckeri, which is taxonomically tentative in the genus *Yersinia*, has been encountered mainly in the United States and Canada as a natural component of fresh water ecosystems. *Y. ruckeri* causes enteric red mouth disease of salmon and fish when fish are exposed to large numbers of bacteria (Ross et al., 1966). The disease is usually enzootic and occasionally epizootic in fish hatcheries especially under conditions of stress or poor environmental factors (Stevenson, 1997). Five serovars and two biotypes of *Y. ruckeri* have been identified. Serovar I strains are usually D-sorbitol positive, while serovar 2 isolates are usually D-sorbitol negative (Stevenson and Daly, 1982; Stevenson and Airdrie, 1984). A 36-MDa transferable plasmid encoding resistance to tetracycline and sulfonamides has been identified in some *Y. ruckeri* strains (DeGrandis and Stevenson, 1985).

Enrichment and Isolation Procedures

Isolation of *Yersinia* strains from noncontaminated normally sterile samples (blood, lymph nodes) can be performed by using blood agar or nutrient agar incubated for 48 h at 28°C, or 24 h at 37°C followed by 24 h at room temperature. The isolation of *Y. pestis* from contaminated samples requires inoculation (subcutaneously or percutaneously) of animals (guinea pigs, mice, or rats). The organism can be recovered postmortem from the spleen, liver, or lymph nodes of the inoculated animals. All other *Yersinia* species can be isolated from clinical or food samples by inoculating standard or special selective media (reviewed by Bottone, 1992), of which cefsulodin-irgasan-novobiocin (CIN) agar (Schiemann, 1979), and virulent *Yersinia* enterocolitica (VYE) agar formulated by Fukushima (1987) seem the most useful. The latter medium was developed because of the growth inhibition of many *Y. bercovieri* and *Y. pseudotuberculosis* strains on CIN agar (Fukushima and Gomyoda, 1986). All these media should preferably be incubated for 48 h at 28–29°C or for 24 h at 37°C followed by 48–72 h at room temperature. Recovery of *Yersinia*

TABLE BXII.γ.284. Correlation of biogroup and serogroup with ecologic and geographic distribution

Biogroup	Associated with human infections	Serogroup(s)	Ecologic distribution
1B	+	O:8, O:4, O:13a, 13b, O:18, O:20, O:21	Environment, pigs (O:8), mainly in the U.S.A.
2	+	O:9, O:5, 27	Pigs, Europe (O:9), United States (O:5, 27), Japan (O:5, 27)
3	+	O:1, 2, 3, O:5, 27	Chinchilla, pigs (O:5, 27)
4	+	O:3	Pigs, Europe, U.S.
5	+	O:2,3	Hare
1A[a]	−	O:5, O:6, 30, O:7, 8, O:18, O:46, nontypeable	Environment, pigs, food, water animal and human feces, U.S.

[a] *Y. enterocolitica* isolates comprising biogroup 1A may be opportunistic pathogens in patients with underlying disorders.

strains from contaminated samples can be improved by various cold enrichment techniques (van Pee and Straiger, 1979; Lee et al., 1980).

Maintenance Procedures

Stab inoculations of *Yersinia* strains in conventional stock culture media stored in the dark at room temperature or at 4°C provide living cultures for 10 years or more, if the tubes are tightly sealed. Lyophilization and deep-freeze storage in 10% glycerol are suitable preservation techniques. To keep a strain fully virulent, it should never be subcultured at 37°C, but always at 25–28°C.

Procedures for Testing Special Characters

Methods to test tetrathionate reduction, tellurite reduction, and the type of nitrate reductase have been described or referenced by Bercovier et al. (1979). The Ca^{2+}-dependency of virulent *Yersinia* strains is evaluated on magnesium oxalate medium[1] (Higuchi and Smith, 1961) as follows. Inoculate 0.1 ml of a bacterial suspension (10^5 cells/ml) onto two plates: one is incubated at 37°C, the other at 26°C. Check colony numbers on the two plates after 2–3 d. Colonies growing at 26°C but not at 37°C are Ca^{2+}-dependent. A fully virulent strain should give confluent growth at 26°C, whereas only 10–100 colonies should appear at 37°C. The autoagglutination test (Laird and Cavanaugh, 1980) to detect virulent *Yersinia* strains is performed by inoculating 10 or more isolated colonies, individually into a pair of tubes (13 × I00 mm) containing 2 ml of RPMI-1640 medium containing 10% fetal calf serum and 25 ml HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). One tube is incubated at 37°C, the other at 26°C. After incubation at 26°C for 18 h, virulent isolates give a uniform turbidity; at 37°C a layer of agglutinated bacteria appears at the bottom of the tube and the overlying supernatant remains clear. Avirulent strains give uniform turbidity at both 26°C and 37°C, and rough strains show spontaneous agglutination at both temperatures.

Differentiation of the genus *Yersinia* from other genera

Characteristics useful for differentiating *Yersinia* from other physiologically similar genera are listed in Table BXII.γ.280, and in Tables BXII.γ.193, BXII.γ.194, and BXII.γ.196 in the chapter on the family *Enterobacteriaceae*.

1. Magnesium oxalate medium consists of blood agar base (BBL, or any other manufacturer if the Ca^{2+} content of the base is low), 40.0 g; distilled water, 830 ml. Sterilize at 121°C for 15 min and cool to 45°C. From stock solution sterilized by filtration, aseptically add the following ingredients: $MgCl_2$ solution (23.8 g/l), 80 ml; sodium oxalate solution (33.5 g/l), 80 ml; and glucose solution (180.1 g/l), 10 ml.

Taxonomic Comments

The genus *Yersinia* was proposed by van Loghem (1944) in order to separate *Y. pestis* and *Y. pseudotuberculosis* (formerly in the genus *Pasteurella*) from *Pasteurella* species *sensu stricto* (i.e., *P. multocida*, etc.), from which they differ in their negative oxidase reaction and in their DNA base composition. The genus *Yersinia* belongs to the family *Enterobacteriaceae*. *E. coli* tDNA (i.e., the genes coding for transfer RNA) and *Y. pestis* tDNA are 63% related (Brenner et al., 1976), a value similar to that found for *E. coli* tDNA and *Hafnia alvei* tDNA. All *Yersinia* species express the common enterobacterial antigen. Their physiological characteristics and their fatty acid content are similar to those of all *Enterobacteriaceae* species. The mol% G + C range of *Yersinia* species is 46–50 and is consistent with that for *Enterobacteriaceae* species.

The genus *Yersinia* currently consists of 11 different species. Based on DNA–DNA hybridization studies, all of these species are more closely related to each other than to any other *Enterobacteriaceae* species (Brenner et al., 1980e; Bercovier et al., 1984; Aleksic et al., 1987; Wauters et al., 1988). The genus *Yersinia* can be considered a very homogeneous taxon.

DNA relatedness among *Yersinia* species is 40% or higher, except for *Y. ruckeri*, which is at most 38% related to other *Yersinia* species. DNAs of *Y. ruckeri* strains have been shown to be 30% related to *Serratia* species (Ewing et al., 1978). *Y. ruckeri* was included in *Yersinia* because its mol% G + C of 48 is closer to that of *Yersinia* species than to that of *Serratia* species. Because the phenotypic characteristics of *Y. ruckeri* are very different from those of other *Yersinia* species (Tables BXII.γ.281 and BXII.γ.282), it might constitute a new genus by itself. Phylogenetic studies would be helpful in clarifying this problem.

Strains of *Y. enterocolitica* belonging to the five different biovars (Table BXII.γ.283), including the metabolically inactive biovar 5 strains, constitute a homogeneous genomospecies (Bercovier et al., 1980a). The strains originally described as *Y. enterocolitica*-like organisms or atypical *Y. enterocolitica* have been separated into three different species: *Y. intermedia* (Brenner et al., 1980a), *Y. frederiksenii* (Ursing et al., 1980a), and *Y. kristensenii* (Bercovier et al., 1980c). *Y. frederiksenii* consists of three genetic groups based on DNA–DNA hybridization (Ursing et al., 1980a). For practical reasons, because there are no phenotypic differences among the three genetic groups, only one species, *Y. frederiksenii*, has been proposed for these rhamnose-positive strains. More study of phenotypic characteristics is needed to separate the three genetic groups.

The DNAs of *Y. pestis* strains, regardless of biovar, and of *Y. pseudotuberculosis* are 90% or more interrelated. This explains the antigenic and biochemical similarities of the two species. Based on DNA data, Bercovier et al. (1980b) proposed that the two

species constitute a single species, divided into two subspecies: *Y. pseudotuberculosis* subsp. *pseudotuberculosis* and *Y. pseudotuberculosis* subsp. *pestis*. Presently, however, the two species remain distinct, largely because of fear of omitting the subspecies epithet subsp. *pestis*, which may present a potential hazard for laboratory-acquired infections (Williams, 1983).

Ursing et al. (1980b) have shown, based on DNA and physiological data, that *Y. philomiragia* (Jensen et al., 1969) is not related to the genus *Yersinia*, and furthermore, that it is not a member of the family *Enterobacteriaceae*. This species has now been assigned to the genus *Francisella* as *F. philomiragia*.

Further Reading

Bercovier, H., J.M. Alonso, Z. Bentaiba, J. Brault and H.H. Mollaret. 1979. Contribution to the definition and taxonomy of *Yersinia enterocolitica*. Contr. Microbiol. Immunol. *5*: 12–22.

Bottone, E.J. 1977. *Yersinia enterocolitica*: a panoramic view of a charismatic microorganism. Crit. Rev. Microbiol. *5*: 211–241.

Bottone, E.J. 1997. *Yersinia enterocolitica*: the charisma continues. Clin. Microbiol. Rev. *10*: 257–276.

Bottone, E.J. 1999. *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbes Infect. *1*: 323–333.

Brenner, D.J., J. Ursing, H. Bercovier, A.G. Steigerwalt, G.R. Fanning, J.M. Alonso and H.H. Mollaret. 1980. Deoxyribonucleic acid relatedness in *Yersinia enterocolitica* and *Yersinia enterocolitica*-like organisms. Curr. Microbiol. *4*: 195–200.

Brubaker, R.R. 1991. Factors promoting acute and chronic diseases caused by yersiniae. Clin. Microbiol. Rev. *4*: 309–324.

Fukushima, H. 1987. New selective agar medium for isolation of virulent *Yersinia enterocolitica*. J. Clin. Microbiol. *25*: 1068–1073.

Kaneko, S. and T. Maruyama. 1987. Pathogenicity of *Yersinia enterocolitica* serotype *O:3*, biotype 3 strains. J. Clin. Microbiol. *25*: 454–455.

Ostroff, S. 1995. *Yersinia* as an emerging infection: epidemiologic aspects of yersiniosis. Contrib. Microbiol. Immunol. *13*: 5–10.

Wauters, G., M. Janssens, A.G. Steigerwalt and D.J. Brenner. 1988. *Yersinia mollaretii* sp. nov. and *Yersinia bercovieri* sp. nov., formerly called *Yersinia enterocolitica* biogroups 3a and 3b. Int. J. Syst. Bacteriol. *38*: 424–429.

Wauters, G., K. Kandolo and M. Janssens. 1987. Revised biogrouping scheme of *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. *9*: 14–21.

Differentiation of the species of the genus *Yersinia*

Characteristics useful in differentiating the various species of *Yersinia* are listed in Table BXII.γ.282.

List of species of the genus Yersinia

1. **Yersinia pestis** (Lehmann and Neumann 1896) Van Loghem 1944, 15[AL] (*Bacterium pestis* Lehmann and Neumann 1896, 194; *Yersinia pseudotuberculosis* subsp. *pestis* Bercovier, Mollaret, Alonso, Brault, Fanning, Steigerwalt and Brenner 1981a, 383.)
pes' tis. L. n. *pestis* plague, pestilence.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. Three biogroups have been described in relation to the geographical distribution of the organism: (a) biogroup antiqua produces acid aerobically from glycerol, reduces nitrate to nitrite, does not ferment melibiose, and is found in Central Asia and Central Africa; (b) biogroup medievalis produces acid from both glycerol and melibiose, but does not reduce nitrate to nitrite; it is found in Iran and the former USSR; and (c) biogroup orientalis (synonym: oceanic) does not produce acid from either glycerol or melibiose but reduces nitrate to nitrite and is distributed worldwide. Some rare atypical strains positive in their reactions for urease and L-rhamnose have been reported. *Y. pestis* is the causative agent of plague. The disease can be reproduced experimentally in mice, rats, guinea pigs, and monkeys.

   The mol% G + C of the DNA is: 46 (T_m).
   Type strain: ATCC 19428, NCTC 5923.

2. **Yersinia aldovae** Bercovier, Steigerwalt, Guiyoule, Huntley-Carter, and Brenner 1984, 171[VP]
al.do' vae. M.L. gen. n. *aldovae* in honor of Eva Aldova, the Czechoslovakian microbiologist who first isolated the bacterium.

   The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. The species is composed of isolates previously referred to as *Y. enterocolitica*-like, group X2 (Bercovier et al., 1980a). Two biovars exist—one typically sucrose negative, and one sucrose positive. Differentiation of *Y. aldovae* from urease-positive *Hafnia alvei* may be achieved by production of gas by *H. alvei* from D-glucose at 36°C and susceptibility to *Hafnia* specific bacteriophage (Guinée and Valkenburg, 1968). *Y. aldovae* has been isolated from aquatic ecosystems—drinking water, river water, fish, and rarely soil. *Y. aldovae* has not been implicated in animal infections and has not been isolated from humans.

   The mol% G + C of the DNA is: 48 (T_m).
   Type strain: ATCC 35236, CNY 6065.
   GenBank accession number (16S rRNA): X75277.

3. **Yersinia bercovieri** Wauters, Janssens, Steigerwalt, and Brenner 1988, 428[VP]
ber.co.vi.e' ri. M.L. n. *bercovieri* in honor of Herve Bercovier, who first described biogroups 3A and 3B and has made outstanding contributions to the taxonomy and ecology of yersiniae.

   Formerly called *Y. enterocolitica* biogroup 3b (Bercovier et al., 1978). Later transferred to *Y. enterocolitica* biogroup 6 (Wauters et al., 1987) because of negative Voges–Proskauer reaction. *Y. bercovieri* must be distinguished from rarely occurring pathogenic strains of serogroup 0:3 that are Voges–Proskauer-negative variants of biogroup 3 (Kaneko and Maruyama, 1987). The species conforms to the definition of the family *Enterobacteriaceae* and the genus *Yersinia*. The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. *Y. bercovieri* strains have been isolated from human stool specimens, animals, raw vegetables, soil, and water. There is no evidence of pathogenicity for humans.

   The mol% G + C of the DNA is: 50 (T_m).
   Type strain: ATCC 43970, CDC 2475-87.

4. **Yersinia enterocolitica** (Schleifstein and Coleman 1943)

Frederiksen 1964, 104[AL] (*Bacterium enterocoliticum* Schleifstein and Coleman 1943, 56.)
en.ter.o.co.li' ti.ca. Gr. n. *enteron* intestine; Gr. n. *colon* of the colon; Gr. suff. *iticos* pertaining to; M.L. fem. adj. *enterocolitica* pertaining to the intestine and colon.

The characteristics are as described for the genus and listed in Tables BXII.γ.281 and BXII.γ.282. The Voges–Proskauer test is usually positive at 22–28°C and negative at 37°C. Biovars of *Yersinia enterocolitica* are listed in Table BXII.γ.283 and, like phagovars and serogroups, are useful epidemiological tools. Rare atypical strains that are either positive for their reactions on Simmons' citrate and for acid production from lactose and raffinose (due to a metabolic plasmid) or negative for urease activity have been reported. When incubated at 20°C, *Y. enterocolitica* strains produce a broad-spectrum mannose-resistant hemagglutinin that is lost at 37°C (MacLagan and Old, 1980). *Y. enterocolitica* causes diarrhea, terminal ileitis, mesenteric lymphadenitis, autoimmune arthritis, abscesses, and septicemia in humans. The disease can be reproduced experimentally in mice, gerbils, and monkeys. Other human infections are listed in Table BXII.γ.285. The species has been isolated from a wide variety of sources in the environment (live and inanimate) including foods and from healthy humans and animals, especially the pig.

The mol% G + C of the DNA is: 48.5 ± 1.5 (T_m, Bd).
Type strain: 161, ATCC 9610, CIP 80-27, DSM 4780.
GenBank accession number (16S rRNA): M59292.
Additional Remarks: The type strain belongs to biovar I B, serogroup 0:8, and phagovar X.

5. **Yersinia frederiksenii** Ursing, Brenner, Bercovier, Fanning, Steigerwalt, Brault and Mollaret 1981, 217[VP] (Effective publication: Ursing, Brenner, Bercovier, Fanning, Steigerwalt, Brault and Mollaret 1980a, 213.)
fred.er.ik.sen' i.i. M.L. gen. n. *frederiksenii* of Frederiksen; named after the Danish microbiologist Wilhelm Frederiksen, who made a substantial contribution to the study of the genus *Yersinia*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. This species is composed of three different genomospecies. One group is positive for β-xylosidase and citrate (Simmons), and the type strain belongs to this group. The other two groups are variable or negative for these tests. More phenotypic studies are needed to differentiate the three groups. Some strains are able to ferment raffinose and lactose when they harbor a metabolic plasmid. *Y. frederiksenii* has been isolated mainly from fresh water sources, fish, foods, and occasionally from healthy or sick humans and animals.

The mol% G + C of the DNA is: 48 (T_m).
Type strain: 6175, CIP 80-29.

6. **Yersinia intermedia** Brenner, Bercovier, Ursing, Alonso, Steigerwalt, Fanning, Carter and Mollaret 1981, 217[VP] (Effective publication: Brenner, Bercovier, Ursing, Alonso, Steigerwalt, Fanning, Carter and Mollaret 1980a, 207.)
in.ter.me' di.a. L. fem. adj. *intermedia* intermediate; here it implies that biochemical reactions of this species seem midway between *Y. enterocolitica* and *Y. pseudotuberculosis*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. Media with a high bile salt content (0.8%) are inhibitory, especially when incubated at 37°C. Some biochemical characteristics (citrate utilization; cellobiose, L-rhamnose, and raffinose fermentation) are always expressed at 25–28°C but are inconstant at 37°C. Either a type A or a type B nitrate reductase is present. Eight biovars have been described (Brenner et al., 1980a) based on the fermentation of melibiose, L-rhamnose, α-methyl-D-glucoside, and raffinose, and on the utilization of citrate (Simmons). Of the strains studied, 96% are positive for at least four of these five tests. *Y. intermedia* has been isolated mainly from fresh water sources, fish, foods, and occasionally from both sick and healthy humans (Bottone et al., 1974; Punsalang et al., 1987).

The mol% G + C of the DNA is: 48.5 ± 0.5 (T_m, Bd).
Type strain: 3953, Bottone 48, Chester 48, ATCC 29909, CIP 80-28.

7. **Yersinia kristensenii** Bercovier, Ursing, Brenner, Steigerwalt, Fanning, Carter and Mollaret 1981b, 217[VP] (Effective publication: Bercovier, Ursing, Brenner, Steigerwalt, Fanning, Carter and Mollaret 1980c, 219.)
kris.ten.se' ni.i. M.L. gen. n. *kristensenii* of Kristensen, named after the Danish microbiologist Martin Kristensen, who first isolated this organism.

The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. Growth is delayed (7 d) when cultures are incubated at 41°C and even at 37°C for some isolates. Some strains utilize citrate (Simmons) after 7-d incubation at 25°C. Most strains produce a "musty" or "cabbage-like" odor when grown on nutrient agar. Some strains produce an enterotoxin (ST) when incubated at 22°C and also at 37°C (Kapperud, 1980). *Y. kristensenii* strains have been isolated mainly from environmental sources such as soil, fresh water, foods, and rarely from healthy human and animal carriers. *Y. kristensenii* may occasionally be associated with human enteritis (Bottone and Robin, 1977), and *Y. kristensenii* isolates may be lethal to mice pretreated with iron dextrin (Robins-Browne et al., 1991).

The mol% G + C of the DNA is: 48.5 ± 0.5 (T_m, Bd).
Type strain: 105, ATCC 33638, CIP 80-30.

TABLE BXII.γ.285. Spectrum of *Yersinia enterocolitica* infections

Type of infection	Manifestation/population
Gastrointestinal	Enterocolitis, predominantly in young children; concomitant bacteremia may also be present in infants
	Pseudoappendicitis syndrome (children older than 5 years; adults), acute mesenteric lymphadenitis; terminal ileitis
Septicemia	Especially in immunosuppressed individuals and those in iron overload or being treated with deferrioxamine; transfusion related (usually leads to septic shock syndrome)
Metastatic	Focal abscesses: liver, kidney, spleen, lung; cutaneous manifestations: cellulitis, pyomyositis, pustules, and bullous lesions; pneumonia, cavitary pneumonia; meningitis; panophthalmitis; endocarditis, infected mycotic aneurysm; osteomyelitis
Postinfection sequelae	Arthritis (associated with HLA-B-27), myocarditis, glomerulonephritis, erythema nodosum

8. **Yersinia mollaretii** Wauters, Janssens, Steigerwalt, and Brenner 1988, 427[VP]
mol.la.re′ti.i. M.L. gen. n. *mollaretii* in honor of Henri H. Mollaret, head of the National *Yersinia* Center at the Pasteur Institute in Paris, for his years of study on the classification and epidemiology in the genus *Yersinia*.

Formerly called *Y. enterocolitica* biogroup 3A (Bercovier et al., 1978). Later transferred to *Y. enterocolitica* biogroup 6 (Wauters et al., 1987) because of negative Voges-Proskauer reaction (typical serotype 0:3 strains are Voges–Proskauer positive). *Y. mollaretii* must be distinguished from rarely occurring pathogenic strains of serogroup 0:3 that are Voges–Proskauer-negative variants of biogroup 3 (Kaneko and Maruyama, 1987). The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. The species conforms to the definition of the family *Enterobacteriaceae* and the genus *Yersinia*. *Y. mollaretii* strains have been isolated from human stool specimens, meat, raw vegetables, soil, and drinking water, and lack virulence markers. There is no evidence of pathogenicity for humans.

The mol% G + C of the DNA is: 50–51 (T_m).

Type strain: ATCC 43969, CDC 2465-87.

9. **Yersinia pseudotuberculosis** (Pfeiffer 1889) Smith and Thal 1965, 220[AL] (*Bacillus pseudotuberculosis* Pfeiffer 1889, 5; *Yersinia pseudotuberculosis* subsp. *pseudotuberculosis* (Pfeiffer 1889) Smith and Thal 1965, 220.)
pseu.do.tu.ber.cu.lo′sis. Gr. adj. *pseudes* false; M.L. fem. n. *tuberculosis* tuberculosis; M.L. gen. n. *pseudotuberculosis* of false tuberculosis.

The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. Some freshly isolated strains may require subculturing before expressing their motility. Strains belonging to serogroup IV are citrate positive (Simmons) and malonate positive. Up to 5% of *Y. pseudotuberculosis* strains have been reported to produce acid from adonitol. Some strains, mostly of serogroup III, produce an exotoxin that differs from the *Y. pestis* toxin. Its biological activity is not well defined. *Y. pseudotuberculosis* is a human and animal pathogen responsible for mesenteric lymphadenitis, diarrhea, and septicemia. The disease can be reproduced experimentally in guinea pigs challenged per os and in mice.

The mol% G + C of the DNA is: 46.5 (T_m).

Type strain: ATCC 29833, DSM 8992, NCTC 10275.

Additional Remarks: The type strain belongs to serogroup I.

10. **Yersinia rohdei** Aleksic, Steigerwalt. Bockemühl, Huntley-Carter, and Brenner 1987, 330[VP]
roh′de.i. M.L. gen. n. *rohdei* of Rohde; named in honor of the late Rolf Rohde, who founded the National Reference Center for *Salmonella* in Hamburg, Federal Republic of Germany, and who made many significant contributions to the diagnostic and serological identification of *Enterobacteriaceae*, especially *Salmonella*.

The characteristics are as described for the genus and listed in Tables BXII.γ.281 and BXII.γ.282. Two biovars are recognized based on positive (biovar 1) or negative (biovar 2) reactions for both raffinose and melibiose. *Y. rohdei* strains were isolated from the feces of dogs and humans and from surface water. The clinical significance of *Y. rohdei* as a diarrheal agent in humans and animals is uncertain.

The mol% G + C of the DNA is: 49.1 (T_m).

Type strain: H271-36/78, ATCC 43380.

Additional Remarks: The type strain was isolated from dog feces in Germany in 1978.

11. **Yersinia ruckeri** Ewing, Ross, Brenner and Fanning 1978, 37[AL]
ru′cker.i. M.L. gen. n. *ruckeri* of Rucker; named after R.R. Rucker, who studied the red mouth disease and its etiological agents.

The characteristics are as described for the genus and as listed in Tables BXII.γ.281 and BXII.γ.282. The cells are 1 × 2–3 μm. Filaments can be seen in old cultures (48 h at 22°C). Colonies on nutrient agar are smooth, circular, and slightly raised. Growth is delayed or inhibited on Salmonella–Shigella agar incubated at 37°C but not at 22°C. Corn oil is hydrolyzed when the test is performed at 22°C but not at 37°C. *Y. ruckeri* is one of the agents responsible for red mouth disease in rainbow trout. The disease can be transmitted experimentally from fish to fish. The organism was initially isolated only in North America, but reports of enteric red mouth disease caused by *Y. ruckeri* have also assumed increasing importance in fish farms in Europe (Home et al., 1984). First isolated in 1959 from a dead muskrat found in a marshy area at the Bear River Migratory Bird Refuge in northern Utah. Other strains have been isolated from water in the same area.

The mol% G + C of the DNA is: 48 ± 0.5 (Bd).

Type strain: ATCC 29473.

GenBank accession number (16S rRNA): X75275.

Genus XLII. **Yokenella** *Kosako, Sakazaki, and Yoshizaki 1985, 224[VP] (Effective publication: Kosako, Sakazaki, and Yoshizaki 1984, 124)*

J.J. Farmer III and Frances W. Brenner

Yoken.el′la. dim. ending *-ella;* Japanese abbreviation *"Yoken"* that stands for the National Institute of Health, Tokyo, Japan; M.L. fem. n. *Yokenella* pertaining to Yoken, the National Institute of Health, Tokyo, Japan where the new group of organisms was recognized and studied.

Rod-shaped cells, conforming to the general definition of the family *Enterobacteriaceae*. Contain the enterobacterial common antigen. Gram negative. Motile, with peritrichous flagella. Facultatively anaerobic. Catalase positive (weak). Oxidase negative. Ferment, rather than oxidize, D-glucose and other carbohydrates. Reduce nitrate to nitrite.

Positive for methyl red, citrate utilization (Simmons), lysine decarboxylase, ornithine decarboxylase, motility at 36°C, and

growth in the presence of cyanide (KCN test), β-galactosidase (ONPG test), tetrathionate reductase, and the fermentation of D-mannitol, L-arabinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, melibiose, D-mannose, D-galactose, L-fucose, and D-gluconate. Produce visible gas during fermentation.

Negative for indole production, Voges–Proskauer, H_2S production (TSI), urea hydrolysis, phenylalanine deaminase, arginine dihydrolase, malonate utilization, gelatin hydrolysis (22°C), lipase (corn oil and Tween 80), DNase, esculin hydrolysis, pectinase, chitinase, β-xylosidase, growth at 4°C, and the fermentation of lactose, sucrose, dulcitol, salicin, adonitol, *myo*-inositol, D-sorbitol, raffinose, α-methyl-D-glucoside, erythritol, D-arabitol, glycerol, and mucate.

Susceptible to nalidixic acid, sulfadiazine, gentamicin, kanamycin, tetracycline, and chloramphenicol (disk diffusion method on Mueller-Hinton agar); **usually resistant to colistin, penicillin, ampicillin, carbenicillin, and cephalothin**; variable susceptibility to streptomycin.

Ecological niches are not known, but has been **isolated from human clinical specimens**, insects, and water. **Probably an opportunistic pathogen** for humans. A **rarely isolated member of the *Enterobacteriaceae*.**

The mol% G + C of the DNA is: 58–59.3 (T_m).

Type species: **Yokenella regensburgei** Kosako, Sakazaki, and Yoshizaki 1985, 224 (Effective publication: Kosako, Sakazaki, and Yoshizaki 1984, 124.)

Further descriptive information

Different names for the same organism It is essential for the reader to understand that three vernacular names (*"Hafnia"* species 3, Enteric Group 45, and NIH biogroup 9) and one scientific name (*Koserella trabulsii*) have been used in the literature for the organism that is known today as *Yokenella regensburgei*. This is explained in the section Taxonomic Comments. Until recently, the names *Koserella* and *K. trabulsii* appeared in the literature, so we have inserted these names in parentheses if they were used in the original article.

Literature Since *Yokenella* and *Koserella* were described in 1984–85, there have been several reports in the literature. In addition to the two genus and two species names, "*Hafnia* species 3", "Enteric Group 45", and "NIH biogroup 9" should also be included in computerized literature searches, although these names are rarely used today.

Sources, strains, collections Strains of *Y. regensburgei* have been isolated from a variety of human clinical specimens and other sources. The original isolates of Kosako et al. (1984) were from human clinical specimens; urine (three isolates), wound (one), and abscess (one), and six isolates from insect intestine. The original collection of Hickman-Brenner et al. (1985a) included the type strain for *K. trabulsii* isolated from a wrist wound of a 28-year-old man. The other isolates were from human wounds (four), sputum (two), throat (one), stool (one), knee fluid (one), well water (one), and unknown (one). Since that original report, the CDC has received 10 additional human strains (three wound, two blood, two sputum, one urine, one lung biopsy, and one unknown). The first blood isolate was from a 35-year-old woman who had a bone marrow transplant. Group G streptococci and *Klebsiella pneumoniae* were also isolated. No further clinical information was given for the remaining strains.

Isolates from other sources Six of the original strains reported by Kosako et al. (1984) were originally isolated by F. Haas (Germany) from the intestine of *Heteroptera* (*Pyrrhcoris apterus*). Cassel-Beraud and Richard (1988) studied stools of 88 insect eating bats (*Chaerephon pumila*), which yielded many different bacterial strains. Strains of *K. trabulsii* were identified among the 20 different species identified, most of which were *Enterobacteriaceae*.

Clinical significance Whether *Yokenella* strains can actually cause infections is still being evaluated. Systematic study and additional case reports are needed. Abbott and Janda (1994a) reported two cases that yielded *Y. regensburgei*; one was a 74-year-old man with a "septic knee", and the other was a 35-year-old immunocompromised woman without overt signs of sepsis but with a positive blood culture. Yague et al. (1989) described two cases (*K. trabulsii*) from Spain. The first case involved an isolate from the stool of a 4-year-old boy. *Campylobacter jejuni* was also isolated and was considered to be the causative agent. The second case involved an isolate from the urine of an 88-year-old man with fever and respiratory problems. The colony count was over 100,000 CFU/ml, but there was no evidence of a urinary tract infection. *K. trabulsii* was not considered significant in either case.

Other literature The literature includes reviews and taxonomic studies of new *Enterobacteriaceae* (Richard, 1989; Gilchrist, 1995; Pokhil, 1996; Aleksic and Bockemühl, 1999), and surveys or comparisons of the family *Enterobacteriaceae* for acid phosphatases (Thaller et al., 1995) and polyamines (Hamana, 1996).

Isolation, identification, culture preservation, phenotypic characterization Strain of *Y. regensburgei* are not difficult to grow, and are typical *Enterobacteriaceae* in most respects. See the chapter on the family *Enterobacteriaceae* for general information on growth, plating media, working and frozen stock cultures, media and methods for biochemical testing, identification, computer programs, and antibiotic susceptibility.

Biochemical reactions and differentiation from other *Enterobacteriaceae* Table BXII.γ.193 in the chapter on the family *Enterobacteriaceae* gives the results for *Y. regensburgei* in 47 biochemical tests normally used for identification (Farmer, 1999). There are no genus- or species-specific tests or sequences for the identification. The best approach is to do a complete set of phenotypic tests (growth on plating media, biochemical tests, antibiogram, etc.) and compare the results with all the other named organisms in the family *Enterobacteriaceae*. This approach is described in more detail in the chapter on the family *Enterobacteriaceae*. The biochemical results of a test strain can be compared to all the organisms listed in Table BXII.γ.193 in that section. Several computer programs greatly facilitate analyzing the results. Biochemically, *Yokenella regensburgei* is closest to *Hafnia alvei*. These two organisms can be differentiated because *Y. regensburgei* is Voges–Proskauer and glycerol negative; citrate (Simmons), cellobiose, and melibiose positive; and resistant to colistin and to the *Hafnia*-specific bacteriophage of Guinée and Valkenburg (1968). It also gives a weak, delayed, catalase reaction. These are in contrast to the reactions of *Hafnia alvei*.

Taxonomic Comments

Today there is general agreement on the use of the names *Yokenella* and *Yokenella regensburgei*. However, the names *Koserella* and *Koserella trabulsii* also appear in the literature. Table BXII.γ.192 in the chapter on the family *Enterobacteriaceae* summarizes how these organisms were discovered, named, and how *Yokenella regensburgei* eventually displaced *Koserella trabulsii*.

Agreement to use *Yokenella* rather than *Koserella* Although the CDC workers did not agree with the manner in which Kosako and Sakazaki (1991) proceeded in trying to solve the nomenclatural problem of priority, they decided to use *Yokenella* and *Y. regensburgei* instead of *Koserella* and *K. trabulsii*. In McWhorter et al. (1991), one of us (JJF) wrote: "We now acknowledge that *Y. regensburgei* has priority over *K. trabulsii* because *Y. regensburgei* was published first and gained standing in nomenclature in the same issue of the *International Journal of Systematic Bacteriology* as *K. trabulsii*. In a nomenclatural sense, *K. trabulsii* should now be considered a 'junior subjective synonym' of *Y. regensburgei* and thus an illegitimate name. We will now use the name *Y. regensburgei* instead of the name *K. trabulsii*."

McWhorter et al., 1991 conceded that only quirks in the validation process had given priority to *Koserella* over *Yokenella*, and agreed that since the names *Yokenella* and *Yokenella regensburgei* were published first and should have appeared first in a validation list, they should be used. Publications, databases, computer programs, and biochemical charts of CDC have reflected this use of *Yokenella* rather than *Koserella*. For example, compare Table 1 of Farmer (1999) that used *K. trabulsii* with Table 1 of Farmer (1995) that used *Y. regensburgei*. This informal acceptance resolved the issue as far as usage; however, only an Opinion of the Judicial Commission in the future can resolve the nomenclatural problem described below.

Nomenclatural problem — do *Yokenella* and *Y. regensburgei* have priority over *Koserella* and *K. trabulsii*? Under the rules of the Bacteriological Code, the answer to this question is unclear. Kosako and Sakazaki (1991) argued clearly and logically for their priority, but this was clearly an opinion of Kosako and Sakazaki, which has no judicial standing under the Bacteriological Code. Unfortunately, they did not end the title of their paper with "Request for an Opinion" as previously agreed to resolve the problem of priority (Kosako et al., 1987). The rules of the Bacteriological Code are interpreted differently by different people, and their application in unique circumstances is also a matter of interpretation. Thus, the question of priority remains as only a small nomenclatural problem because the matter of usage was settled in the early 1990s.

Further Reading

Abbott, S.L. and J.M. Janda. 1994. Isolation of *Yokenella regensburgei* (*Koserella trabulsii*) from a patient with transient bacteremia and from a patient with a septic knee. J. Clin. Microbiol. *32*: 2854–2855.

Hickman-Brenner, F.W., G.P. Huntley-Carter, G.R. Fanning, D.J. Brenner and J.J. Farmer. 1985. *Koserella trabulsii*, a new genus and species of *Enterobacteriaceae* formerly known as enteric group-45. J. Clin. Microbiol. *21*: 39–42.

Kosako, Y., R. Sakazaki and E. Yoshizaki. 1984. *Yokenella regensburgei* gen. nov., sp. nov.: a new genus and species in the family *Enterobacteriaceae*. Japan J. Med. Sci. Biol. *37*: 117–124.

List of species of the genus Yokenella

1. **Yokenella regensburgei** Kosako, Sakazaki, and Yoshizaki 1985, 224[VP] (Effective publication: Kosako, Sakazaki, and Yoshizaki 1984, 124.)
re.gens.bur' ge.i. M.L. gen. *regensburgei* pertaining to Regensburg, Germany, where the type strain of *Y. regensburgei* and five other strains in the original paper (Kosako et al., 1984) were isolated from insects.

   The characteristics are as given for the genus. Isolated from human clinical specimens (blood, urine, feces, throat and sputum, wounds, abscess, and others), insect intestines, and well water. Its clinical significance is not known, but if it is pathogenic for humans it is probably a very weak pathogen. However, it is also possible that it is colonizing rather than infecting body sites that are normally not sterile. There is no evidence that it can cause diarrhea or intestinal infections. It should be considered a rarely isolated species of *Enterobacteriaceae* that is probably an opportunistic pathogen for humans. The type strains was isolated by F. Haas from the intestine of an insect (*Pyrrhocoris apterus*) in Regensburg, Germany.

   The mol% G + C of the DNA is: 58–59.3 (T_m).

   Type strain: ATCC 49455, CDC 3020-86, NIH 725-83, JCM 2403.

   Additional Remarks: The American Type Culture Collection includes the five strains of *Y. regensburgei* that were originally deposited by Hickman-Brenner et al. (1985a) as *Koserella trabulsii*. Four were from human clinical specimens and one was from well water.

Order XIV. **Pasteurellales** *ord. nov.*

George M. Garrity, Julia A. Bell and Timothy Lilburn

Pas.teu.rel.la' les. M.L. fem. n. *Pasteurella* type genus of the order; *-ales* suffix to denote order; M.L. fem. n. *Pasteurellales* the *Pasteurella* order.

The order *Pasteurellales* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the order contains the family *Pasteurellaceae*.

Description is the same as for the family *Pasteurellaceae*.

Type genus: **Pasteurella** Trevisan 1887, 94 (Nom. Cons. Opin. 13, Jud. Comm. 1954b, 153.)